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ESSENTIALS OF DEMOGRAPHICAL STUDIES IN CANCER.

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DEVELOPMENTS in recent decades have made the term "Cancer Statistics" inadequate for the description of the ways in which statistics have contributed to cancer research. It is time that this term was reserved for statistical treatment of results, while statistical research into the occurrence of human cancer, be it from racial, genetical, geographical, occupational or other sociological points of view, should be included in the term "Cancer Demography." This will be the case in the following.

Unlike many other branches of science joining in the research of cancer at some stage of its development, cancer sociology can point to a long, though most often neglected, history. When Percivall Pott, in 1775, described the chimney sweep's cancer, not only giving its clinical features, its treatment, and its social aetiology, but also taking active steps toward its prevention, in which we have finally succeeded, it was the first step in cancer sociology. Pott's observation of the latent period of scrotal cancer in chimney sweeps contained a very obvious clue to the experimental production of cancer, but experimental workers seem to have been too fascinated by experimental and pathological problems to take a lesson, not to speak of inspiration, from clinical or sociological observations, and 140 years passed before cancer was provoked experimentally by tar and soot. However, it seems still more surprising that after this experience we have spent decades since Young and Russell's demonstration in 1927 of a causal relationship between alcoholic consumption and cancer of the upper digestive tract in examining the carcinogenicity of numerous other compounds, while alcohol has been examined only in a single experiment on a small scale (Krebs, 1928). Similarly it is well known that the first birth increases the possibility of a later cervical cancer, but where are experiments based on this and similar facts ?

It would not seem unlikely that the slow progress in obtaining results of practical value in the fight against cancer is to a large extent due to our theoretical way of tackling problems. For instance, it is not unlikely that the future will reveal very interesting facts with regard to the influence of cosmic radiation on the incidence of cancer in heavily inbred animals of some kind, but there is considerable evidence that unless the energy released by such radiation shows a

different affinity to individuals of the various social classes, there must be social factors of a far more decisive character influencing the incidence of cancer in man.

In order to bring about a closer contact between cancer research and problems of clinical importance, a more thorough investigation into the aetiology of cancer should be attempted by cancer demography through mass observations. However, it is in the nature of statistics that they cannot provide biological proofs, but only point to correlations and possible correlations, excluding some aetiological possibilities and stressing others. But final aetiological proofs are nearly always provided experimentally. So unless experimental research will devote more of its comparatively rich resources to assisting in cancer demography we shall continue to frustrate the fundamental question of the clinical aetiology of cancer. After all, one is justified in saying that the prevention of the various cancers due to Roentgen radiation, butter-yellow, aniline dyestuffs and lubricating oils, etc., in which we have so far succeeded, is at least as valuable to the unknown individual concerned as the cures obtained by million volt plants, betatrons and the like, although the latter seem to appeal more to the public and to benefactors, and perhaps even to experimental research workers and practitioners.

A second limitation to the statistical part of cancer demography is its dependence on the vital statistics of the area concerned, since the frequency of a disease cannot be estimated without considerable knowledge of the normal population in the area. It is fundamental, but often neglected, that all statistics on cancer cases must be divided up according to race and sex, but it is just as important to know the age distribution of the population examined, preferably on quinquennial age groups, because the various cancers greatly increase with age and at different rates. Subdivisions according to occupation are also important, but they are more difficult to provide than is generally realized, first because of the difference in age distribution between the various occupations, and secondly because half of the cancer cases may occur among people retired from their original work. If it is added that it should be possible to distinguish between material originating from urban and from rural areas it will give some idea of the demands on statistics by medical research, since all these subdivisions should be available at the same time, and naturally each within the others.

In consequence of these extensive demands a close collaboration with the public statistical services involved is vital to the development of good statistics in respect of cancer research. However, in most countries civil servants will think and work very differently from biological research workers with their eternal endeavour to raise new problems and change methods. To this we may add a frequent tendency among the former to take a more national and less universal point of view than is common so far in the medical world. But there is no way out of such diffiulties except the urging the necessity of providing the figures desirable for medical observations on the highest administrative level. Possibly an international authority, for instance under the United Nations, would be the best means to establish international collation of medical statistics in a field as specialized as cancer demography.

Difficulties on the medical side are no smaller, but of a different nature. Most statistical investigations on cancer demography have so far been based on death certificates, and with the progress of therapy this is becoming increasingly inadequate for research purposes with regard to the incidence of cancer. It is

true that progress in diagnostic methods has made death certificates more reliable with regard to certainty of type and site of tumour, although reliability varies with the site, but there is no doubt that the quality of diagnosis varies considerably between countries, and many comparisons carried out in the past must now be considered as unwarranted. An example of this is the variation in the proportion of gastric cancer in different countries, which has been discussed more often than the possible sources of error involved. For instance a first-class textbook of pathology states: "The incidence of gastric cancer in Sweden and Czechoslovakia apparently is about three times that of England. No satisfactory explanation has been offered for these interesting findings" (Anderson, 1948) As far as the present author has been able to establish, the reason for some of the differences may be that death certificates in the thinly populated areas of Northern Sweden for practical reasons are issued by parsons. Furthermore, it will appear from the demands already mentioned that a direct comparison of percentages without a further analysis with regard to age distribution is unsatisfactory, especially in the case of a site such as the stomach, in which the establishment of a definite diagnosis is difficult. Admittedly, death certificates have in the past rendered valuable information, especially in the hands of the English Registrar-General, but this is no guarantee that death certificates from any country will produce similarly reliable details.

It is vital to the use of death certificates that they are referred to the domicile of the patient, and not to the locality where death has occurred. And it must also be remembered that occupational statistics based on death certificates theoretically demand that the occupation of the deceased person should be stated from the same source as in the census, that is, some registration office, and not from the case-record or the statement of relatives, although this might not cause serious errors.

Comparisons of death certificates of different date are often made without the necessary regard to the dangers of this method. Pedigrees of greater length may for instance be misleading because the frequency of cancers of various sites, whether apparent or real, will differ from one generation to the next.

For sites of cancer where therapy is improving death certificates are decreasing in value as indicators of frequency, while other sites may show an apparent increase in frequency due to progress in diagnostic technique, and post-mortem figures will also be subject to this influence. Heady and Kennaway (1949) suggest that the proportional occurrence of lung cancer among the post-mortem total will serve as an indicator of changes in the incidence among the whole population. Such figures are, however, influenced not only by the experience and information available to pathologists, but also through progress in the therapy of early complicating pneumonias and of lung cancer itself.* It is generally assumed that progress in diagnostic technique will cause an increase in the number of cancer cases registered, but according to the author's experience gastric cancer tends to be overestimated, and will often decrease in frequency with improvements in medical facilities. Consequently, comparisons between different countries or periods based on death certificates as well as on other sources of medical information demand additional knowledge of a more direct character.

* Readers of their review are recommended to compare the order of magnitude of the yearly totals criticized with those accepted as a basis for considerations.

For years hence, however, death certificates are bound to play an important part in demography of cancer, because they will be the best information available for the many cases not treated in hospital. Even in the case of compulsory registration there will remain a number of notifications from general practitioners amounting in quality to little more than death certificates. It is also important to the statistician to realize that the difference in quality of diagnosis between hospitals and general practice will cause a difference in the material, unless practically all cases are examined in hospitals, and this will be the case for only very few sites of cancer. Another difference between these groups will appear in the age distribution curves, which must be based on the age at onset of the cancer, theoretically at the onset of symptoms. Accurate statements on this item are unfortunately very difficult to obtain on death certificates, and for hospitals it is most practical to state the date of the first admission, which has the advantage of being an accurate reality.

For all these reasons it will be evident that complete statistics on the incidence of cancer in a given area should contain figures for the number of cases treated in hospital for each site of cancer, and separate age distribution figures for this part of the material as well as for the total. It is not uncommon for authors dealing with a single site of cancer to leave out the total cancer incidence in the population concerned, but this is not permissible when we do not know the interrelations between cancers of different sites. The percentage of cases examined histologically or by autopsy should also be given for each site as an indicator of the basis of the diagnosis, and is particularly valuable in statistics giving figures for diagnoses of a histological character. At the moment such figures can only comprise a part of the cases really occurring, and it is most important to know the actual number of microscopical examinations from which they are collected, even in the case of a diagnosis such as "sarcoma," which sometimes may be founded only on a macroscopical examination.

Unfortunately, many publications on cancer demography are inadequate with regard to statistics, and amount to little more than a summary of the author's own conclusions, although they should contain all the basal figures for both the normal and the cancer populations, as well as the formulas applied, not only with regard to an independent judgment by contemporary workers, but also with a view to retrospective work in the same field in future. Probably it is mostly editors and not authors who are to blame for such omissions, and the same applies to diagrams, which are as essential to such articles as are photomicrographs to histological or roentgenograms to radiological publications. Cost will probably also be blamed for the fact that most of the valuable material on the occurrence of cancer is hidden away in statements from public health authorities. A unification of publications in this field, so that they could be collected in journals accessible to workers in experimental cancer research, would contribute far beyond its cost to the vital connection between experimental research and cancer demography.

But it is most important in such publications that the author ascribes his findings to factors of some established certainty, and is reticent in advancing new theories of his own on the aetiology of cancer. Readers may find, even in modern publications of some standing, that the well-established connection of alcoholic consumption with the incidence of certain cancers is paralleled by the influence of tinned or fried food. A more exact distinction between hypotheses,

theories and proofs would certainly be the best possible contribution available at the moment in this field of research.

The demands of the present paper may appear theoretical and too strict, and it may be said with justification that even important previous publications do not always fulfil them. This has been the case where the biological differences were sufficiently pronounced to be demonstrable even in less perfect materials, but if we want to make progress we must improve our statistical technique, which in cancer demography seems to be unnecessarily far behind cancer therapy. As it has been said, it is not the amount of statistical material but the care with which it is collected and worked out that matters most.

On the other hand, it is the experience of the present author that the more refined statistical methods as a rule will require materials of a more detailed accuracy than that obtainable in cancer demography at its present stage. Furthermore, the statistical treatment should not be allowed to override well-founded medical conclusions. An example may serve to illustrate this point. For the years 1942–44 the age distribution curve for the incidence of mammary cancer in Danish hospitals showed a downward turn from the 47th to the 57th year, and this decrease proved statistically significant. Trusting this significance, workers in England, Switzerland and other countries took the trouble to examine their material divided up into sufficiently small age classes, and found a similar trend, though within the limits of the standard error; when the Danish material was extended to comprise the years 1942–46 the downward turn of the curve changed to a level, and was no longer of statistical significance as a downward turn. There is, however, no reason to doubt the medical significance of this sudden change at the climacteric age, whatever it may amount to.

At the present moment we should be satisfied if we can establish fairly reliable registration of cancer cases in different parts of the world and on a comparable basis. Provided with a good system of death registration, it will be possible to do this mainly on the basis of hospital statistics and cases from private clinics, supplemented by death certificates. Repeated articles keeping doctors informed on the work being carried out and some token registration fee are, in the author's experience, efficient means for convincing doctors that they have not fallen victims to bureaucratic caprices, but are contributing to active sociological research, which is usually appreciated by them. Even if it should take some time to persuade other bodies to support the scheme, cancer demography should carry on, seeking its justification in the face of experimental research through the proverbial interrelation of their respective final aims: prevention and cure.

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CANCER OF THE MOUTH: A REPORT ON 800 CASES.

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THE year 1936 was chosen for the start of this investigation, since it was at this time that the 5-gramme teleradium unit came into operation. Cases seen between January, 1936, and December, 1945, have been analysed so that a minimum 3-year follow-up is possible. Patients referred to us with recurrence after treatment elsewhere are excluded, though the numbers are noted for the sake of interest.

The majority of cases are hospital patients, but private patients are also included. All patients were seen by both surgeons and radiotherapists, and at the original consultation a "*First Planned Treatment*," which often utilized several different methods, was decided upon. Later in this paper an attempt is made to assess the success or failure of this first treatment in terms of recurrences.

GENERAL REVIEW OF 800 CASES.

In the ten-year period 1936–1945 inclusive 949 cases of buccal cancer were seen. Of these, 149 were referred to us as recurrences, or after the commencement of treatment elsewhere, and have therefore been excluded. This leaves 800 cases for analysis.

The primary site.

It is possible to classify the primary sites in which buccal neoplasms occur in a number of ways. Anatomically the dividing line between the mouth and the oro-pharynx lies along the anterior faucial pillars, the soft palate and the tongue in the region of the circumvallate papillae. Growths of the tonsil and the posterior third of the tongue therefore belong anatomically to the oro-pharynx, but in this paper they have been included with the mouth. So also have growths of the lips. Those of the vallecula and epiglottis are excluded.

From the clinical aspect of the disease, neoplasms of certain sites have affinities which seem to be of more importance than their anatomical position. It is, for instance, often difficult to say whether a growth belongs to the floor of the mouth or the inferior surface of the tongue, though both behave in much the same way. It may be impossible to be sure whether cancer which is advanced when first seen has arisen from the tonsil proper or in the region of the anterior faucial pillar. Yet the clinical course of the two conditions is very different.

Therefore the grouping shown in Table I has been adopted, since it seems to satisfy the clinical and therapeutic aspects most conveniently.

Table I shows the number of cases included in each site and the sex ratio. In this series buccal cancer was approximately ten times as common in men as in women.

TABLE I.—*The Cases by Sites.*

Primary sites.	New cases (total 800).	Recurrent cases (total 149).	Sex. Male.	Sex. Female.
1 Mucosal surfaces of lips	160	35	158	2
2 Mucosa of upper alveolus and hard palate	40	9	32	8
3 Mucosa of lower alveolus	54	9	44	10
4 Buccal mucosa	59	7	54	5
5 Floor of mouth and inferior surface of tongue	136	19	130	6
6 Dorsum and borders of anterior two-thirds tongue	150	32	115	35
7 Posterior third tongue (and whole tongue)	75	15	70	5
8 Anterior faucial pillar and soft palate	85	15	83	2
9 Tonsil proper	41	8	34	7

The age and duration of symptoms.

It is well recognized that buccal cancer affects a relatively old group of patients and this series is no exception. This is the first fact that militates against long survival periods. It has not been so generally emphasized, however, that the duration of symptoms before treatment is often disconcertingly short. In spite of this many cases were of an advanced character, i.e. stages III or IV, when first seen. This is the second difficulty which makes the prognosis so comparatively bad.

Table II illustrates the age groups into which the patients fitted. None was under 35, and the oldest (a man with carcinoma of the lip) was 104.

TABLE II.—*Age Groups.*

Age.	Number of cases (total 800).
Under 40	14
40–50	60
51–60	179
61–70	320
71 and over	225
Unrecorded	2

Table III shows the duration of symptoms before the commencement of treatment. This did not vary much according to the site of the primary lesion. Patients with visible growths on the lip or front of the tongue did not attend for advice any sooner than those whose growths were situated further back, and in whom it was often the cervical metastases which were first noticed. It will be seen that two-thirds of the patients had a history of less than 6 months. This is in agreement with the figures of other investigators.

TABLE III.—*Duration of Symptoms before Treatment.*

Duration of symptoms.	Number of cases (total 800).
Less than 3 months	224
3–6 months	307
7–12 months	150
1–3 years	68
More than 3 years	30
Uncertain	21

The staging of the disease.

Not all authorities agree on the staging of cancer of the mouth, although the presence or absence or regional lymph nodes is of course recognized as being of signal importance with regard to prognosis and treatment. We have adopted the following staging, which is similar to that employed at the Christie Hospital and Holt Radium Institute, Manchester:

Stage I.—Primary growth limited in extent to site of origin. No loss of mobility or function. No palpable nodes.

Stage II.—Primary growth spreading to adjacent structures. No palpable nodes.

Stage III.—Primary growth as in Stage I or II. Regional lymph nodes invaded, but mobile and confined to area of immediate lymphatic drainage (i.e. operable).

Stage IV.—Primary growth too advanced for any treatment. Lymph nodes fixed, matted or bilateral, or distant metastases present.

Table IV shows, as already mentioned, how many cases had already reached Stages III or IV when first seeking treatment. The lip, which is a notable exception, is charted separately. The fact that patients with cancer of the lip did not seek advice any sooner than those with growths at other sites confirms that cancer of the lip metastasizes more slowly than cancer inside the mouth.

TABLE IV.—*The Cases by Stages.*

Stage.	Number of cases.	
	All sites except lip (total 640).	Lip (total 160).
I	129	114
II	134	6
III	213	29
IV	162	11
Unstaged	2	0

Since the presence of involved lymph nodes so materially alters the prognosis it is convenient to summarize Table IV in the following manner:

Stage.	All sites except lip.	Lip.
I and II	263 (41%)	120 (75%)
III and IV	375 (59%)	40 (25%)

Histology.

Since the majority of the cases were treated by radiotherapy the whole neoplasm was very seldom available for histological study, but a biopsy was performed in about 4 out of every 5 cases, and the diagnosis of malignancy confirmed.

Broders' (1920) grading of neoplasms was given up several years ago at the Royal Cancer Hospital. It was recognized that wide variations in the type of cell could be encountered in different sections from the same growth, and that for this reason alone attempts at the estimation of the degree of differentiation from the biopsy of a small piece of tissue were likely to be not only of no value, but actually misleading.

Of the 800 cases under review a biopsy was performed in 639. The histology was as follows:

Squamous carcinoma . .	631
Lymphoepithelioma . . .	4
Basal-celled carcinoma . .	1
Malignant sialoma . . .	2
Lymphosarcoma . . .	1
	639

Wassermann reaction.

This was performed on 500 out of the 800 cases. There were 79 positive and 421 negative results—a proportion of 16 per cent positive reactions. Growths of the tongue were most frequently associated with a positive Wassermann reaction, and in those patients upon whom the test was performed 28 per cent of positive reactions were recorded. Patients with cancer of the buccal mucosa had positive Wassermann reactions in 20 per cent of cases, while those with cancer of the palate and alveoli were seldom found to be positive (3 per cent and 0·5 per cent respectively).

Multiple primaries.

In 1932 Lord Moynihan recorded a case where a second primary had appeared in the mouth some years after the first growth had been successfully treated, and he added that he had never seen this occur before (Moynihan, 1932). This stimulated the collection of such cases, and Fraser (1931) recorded 5 multiple primaries in 105 cases of buccal cancer. It is now well recognized that such double or even triple primary growths may occur in different positions in the buccal cavity and upper air passages. Warren and Gates (1932) investigated the problem in considerable detail, and concluded that a patient with one cancer was statistically more likely to develop another cancer than a normal individual. Amongst their own cases they found 3·7 per cent of multiple primaries in 1078 patients dying from cancer. Watson (1939) calls attention to the fact that when a patient with carcinoma of the oesophagus has a second growth this is nearly always situated in the mouth. The corollary is also true, that when the first growth is in the mouth the second is very frequently in the upper digestive tract.

Amongst this series of 800 cases there were 30 patients who developed a second primary (3·75 per cent). The following criteria have been adopted for calling a neoplasm a new primary. It is recognized that these are not so strict

as those proposed by other authorities, e.g. originally by Billroth (1889), but they seem to stand the test of practical experience.

Each growth must be distinct, and if arising in the same anatomical site (e.g. the buccal cavity) there must be no possibility of the one being a local recurrence of the other. Each growth must be proved histologically, and if the histology is the same (e.g. squamous-celled) then the possibility of metastasis must be reasonably excluded.

Table V shows the distribution of the 30 cases. These are grouped according to the site of the first and the subsequent growths. It will be seen that the majority of the latter occurred in the digestive tract, and most often between the lip and the cardiac sphincter of the stomach.

TABLE V.—*Multiple Primaries.*

First primary.		Subsequent primary.		
		Lip—cardia.	Cardia—rectum.	Other sites.
Lip	16	8	1	7—6 skin 1 bladder
Tongue	4	1	2	1— bladder
Rest of mouth	10	3	2	5—1 skin 1 bronchus 1 nasopharynx 1 pyriform fossa 1 larynx
Total	30	12	5	13

RESULTS OF TREATMENT.

As previously stated, the majority of the cases were treated by radiotherapy. Many had a combination of different types of therapy, but it has been possible to analyse two reasonably large groups in detail—those treated by teleradium, occasionally assisted by a radium applicator, needles or radon seeds, and those treated by X-ray therapy.

Teleradium.

The 5-gramme unit was used for the most part. A few cases were treated, mainly palliatively, with the 1-gramme unit. It will be observed in the account which follows that interstitial radium has been comparatively neglected. This was a deliberate decision made in order to determine the value of teleradium therapy.

X-ray therapy.

This has included high voltage, medium voltage and intra-oral contact therapy. 400 kV. and 200 kV. tubes have been used to give external irradiation to the primary tumour and nodes. Usually the dose to the primary tumour has been increased by intra-oral contact therapy with a low voltage tube (140 kV., 60 kV., or 45 kV.). For Stage I and II cases, particularly cancer of the lip, low voltage contact therapy has sometimes been the only method of X-ray therapy employed.

In order to simplify the detailed tables of results, the primary sites have been condensed as follows :

Lip		160 cases (Table VI)
Anterior two-thirds of tongue		150 cases (Table VII)
Other buccal sites :		
Upper alveolus and hard palate	40	289 cases (Table VIII)
Lower alveolus	54	
Buccal mucosa	59	
Inferior surface of tongue and floor of mouth	136	
Oro-pharyngeal sites :		
Posterior one-third of tongue	75	201 cases (Table IX)
Anterior faucial pillar and soft palate	85	
Tonsil	41	
		800 cases

A simplified table indicating the survival rates for each individual site is given later (Table X).

Definitions of terms used.

D.I.D.—Dead of intercurrent disease without cancer present.

Net number.—The number of patients treated, less those untraced and those dead of intercurrent disease.

Dead of cancer.—Includes also those dead from unknown cause, intercurrent disease with cancer present, or following treatment, in most cases within three months of the completion of treatment.

Net survival rate.—The percentage of the net number of patients alive with or without cancer.

Absolute survival rate.—The percentage of the total number of new patients, including those not treated, who are still alive. This gives a true statistical picture of what becomes of all the patients who attend.

TABLE VI.—*Lip.* 160 *cases*—4 *not treated.*

	Cases seen from 1936–45 inclusive : state at the end of 3 years, 156 cases.						1936–43 inclusive : 5 years, 122 cases.		1936–38 inclusive : 10 years, 42 cases.
Method of treatment :—	Radium.		X-rays.		All methods.*		All methods.		All methods.
Stage :—	I and II	III and IV	I and II	III and IV	I and II	III and IV	I and II	I II and IV	All stages.
Number treated	39	10	74	21	120	36	93	29	42
Untraced	1	2	12	1	14	3	21	3	9
D.I.D.	2	—	6	2	9	2	10	1	9
Net number	36	8	56	18	97	31	62	25	24
Dead of cancer	6	5	5	12	11	18	12	18	14
Alive	30	3	51	6	86	13	50	7	10
Net survival rate	83%	—	91%	—	89%	42%	81%	28%	42%
Absolute survival rate		—		—	62%		46%		23%

* Including 12 treated by combined methods.

The lip—160 *cases.*

Of the 156 treated cases, 26 were treated by a radium applicator, 22 by teleradium with or without an applicator, 1 by radium needles, 95 by X-rays, and 12 by combined methods (Table VI).

The early cases responded best to X-ray therapy, and with this treatment a 3-year net survival rate of 91 per cent was obtained for Stage I and II cases. The considerable difference between the 3-year net and absolute survival rates is due to the rather large number of untraced cases.

Anterior two-thirds of tongue—150 *cases.*

Only those neoplasms occurring on the dorsum and lateral border of the tongue anterior to the circumvallate papillae are included in this group.

In this instance it is possible to compare the results of three methods of treatment:

Radium.—83 cases. Teleradium only was used in 67 cases, teleradium and interstitial needles in 12, needles only in 4. Many Stage I and II cases received what amounted to prophylactic irradiation of the lymphatic areas of the neck. It has already been stated that interstitial radium therapy was seldom used in order to give teleradium an extensive trial.

X-rays.—33 cases.

Surgery only.—14 cases. The method involved was usually diathermy excision of the primary neoplasm or of the anterior portion of the tongue, together with block dissection of the neck, either initially or at a later date when enlarged lymph nodes appeared.

Combined methods.—In addition combined treatments were given to 17 patients. Only 16 block dissections were performed in the whole group of 150 cases.

Table VII shows that in early cases surgery gave the best results (6 out of 7 living three years). The overall 3-year net survival rate for all methods of treatment was 30 per cent, the 5-year rate 20 per cent, and the 10 year rate 14 per cent.

TABLE VII.—*Anterior Two-thirds of Tongue.* 150 *cases*—3 *not treated.*

	Cases seen from 1936–45 inclusive:— state at the end of 3 years, 147 cases.								1936–43 inclusive: 5 years, 128 cases.		1936–38 inclusive: 10 years, 42 cases.
Method of treatment:—	Radium.		X-rays.		Surgery only.		All methods.*		All methods.		All methods.
Stage:—	I and II	III and IV	I and II	III and IV	I and II	III and IV	I and II	III and IV	I and II	III and IV	All stages.
Number treated	43	40	15	18	9	5	77	70	66	62	42
Untraced	2	—	—	1	—	1	2	2	4	2	1
D.I.D.	4	1	—	—	2	—	8	1	9	2	5
Net number	37	39	15	17	7	4	67	67	53	58	36
Dead of cancer	22	38	7	13	1	4	34	60	36	53	31
Alive	15	1	8	4	6	—	33	7	17	5	5
Net survival rate		—		—		—	49%	10·5%	32%	9%	14%
Absolute survival rate		—		—		—		27%		17%	11%

* Including 17 treated by combined methods.

The remainder of the buccal cavity—289 *cases.*

This group includes neoplasms arising from the inferior surface of the tongue, floor of mouth, the mucosa covering the alveoli and the hard palate and that of the inner aspect of the cheek.

In the 163 cases treated with radium, teleradium only was used in 140 cases, teleradium combined with needles or an applicator in 17, and needles or an applicator in 6.

The results of radium treatment for Stage I and II cases are seen to be statistically better than those of X-ray treatment (Table VIII).

TABLE VIII.—*Upper Alveolus and Hard Palate, Lower Alveolus, Buccal Mucosa, Floor of Mouth and Inferior Surface of Tongue.* 289 *cases*—9 *not treated.*

	Cases seen from 1936–45 inclusive:—state at the end of 3 years, 280 cases.						1936–43 inclusive: 5 years, 220 cases.		1936–38 inclusive: 10 years, 83 cases.
Method of treatment:—	Radium.		X-rays.		All methods.*		All methods.		All methods.
Stage:—	I and II	III and IV	I and II	III and IV	I and II	III and IV	I and II	III and IV	All stages.
Number treated	63	100	41	47	121	159	86	134	83
Untraced	4	7	2	2	8	9	6	7	5
D.I.D.	3	1	2	3	5	6	7	8	8
Net number	56	92	37	42	108	144	73	119	70
Dead of cancer	20	77	25	36	50	120	45	103	64
Alive	36	15	12	6	58	24	28	16	6
Net survival rate	—		—		54%	16·5%	38%	13·5%	8·5%
Absolute survival rate	—		—		28%		19·5%		7%

* Including 29 treated by combined methods.

Oro-pharyngeal sites—201 *cases.*

This group has already been defined. Cases in which the whole tongue was involved have been included here with the posterior third of the tongue.

TABLE IX.—*Posterior One-third of Tongue* (*including Whole Tongue*), *Anterior Faucial Pillar and Soft Palate, Tonsil.* 201 *cases*—12 *not treated.*

	Cases seen from 1936–45 inclusive:—state at the end of 3 years, 189 cases.						1936–43 inclusive: 5 years, 147 cases.		1936–38 inclusive: 10 years, 55 cases.
Method of treatment:—	Radium.		X-rays.		All methods.*		All methods.		All methods.
Stage:—	I and II	III and IV	I and II	III and IV	I and II	III and IV	I and II	III and IV	All stages.
Number treated	33	70	13	49	58	131	44	103	55
Untraced	—	2	—	3	—	5	1	5	2
D.I.D.	5	3	1	2	6	5	9	5	5
Net number	28	65	12	44	52	121	34	93	48
Dead of cancer	15	52	6	39	27	102	22	86	41
Alive	13	13	6	5	25	19	12	7	7
Net survival rate	—		—		48%	15·5%	35%	7·5%	14·5%
Absolute survival rate	—		—		22%		12%		11·5

* Including 24 treated by combined methods.

Radium.—103 cases. In the 103 cases treated with radium, teleradium only was used in 94, needles gave additional irradiation in 8 others, and 1 was treated solely by needling.

X-rays.—62 cases.

Although the numbers are small, Table IX suggests that the early cases responded equally well to X-ray therapy as to radium.

Results for individual sites.

It is of some interest to compare the response to treatment of each of the eight individual sites in the buccal cavity and oro-pharynx (the lip having already been considered). In order to keep Table X within comprehensible limits only the net survival rates are given. The numbers of cases for each particular site can be found by reference to earlier tables. The average figures are surprisingly uniform, namely, about 30 per cent 3-year, 20 per cent 5-year, and 10 per cent 10-year net survivals.

TABLE X.—*All Sites : Net Survival Rates, 3, 5 and 10 years. 640 cases—24 not treated.*

Method of treatment:—	1936–45: 3 years. Radium. %	1936–45: 3 years. X-rays. %	1936–45: 3 years. All methods. %	1936–43: 5 years. All methods. %	1936–38: 10 years. All methods. %
Upper alveolus and hard palate	43	20	30·5	19·5	10
Lower alveolus	28	31	30	27	16·5
Buccal mucosa	36	6	29·5	16·5	10
Floor of mouth and inferior surface tongue	34	30	36·5	25	3
Anterior two-thirds tongue	21	37	30	20	14
Posterior one-third and whole tongue	30	17	21·5	15	12
Anterior pillar and soft palate	28	20	27	13	11
Tonsil	24	28	29·5	19	23
All sites	29	24·5	30	20	11·5

Block dissection.

The total number of block dissections performed was very small. During the period under review the idea of prophylactic block dissection was being abandoned, and operation performed only when operable lymph nodes were

TABLE XI.—*Block Dissections.*

Site:—	Lip.	Anterior two-thirds of tongue.	Other sites.	Total.
Number	7	16	18	41
Operation part of first planned treatment	2	10	10	22
Operation part of subsequent treatment	5	6	8	19
Section positive	4	9	12	25
Section negative	3	7	6	16
Operation deaths	0	2	3	5=12%

palpable. Since only a few of these operations were on patients who had had no previous irradiation to the neck, it is not possible to contrast the relative efficiency of surgery and radiation as a means of treating secondary lymph nodes.

Evaluation of First Planned Treatment.

We have already stated that a scheme of treatment is decided upon when the patient is first seen. It is therefore of interest to know how often this treatment succeeds or fails in its objective, and in the latter case, when there is a recurrence, what are the chances of further arresting the disease.

These facts cannot be determined with any real accuracy, since each case clearly has to be assessed on its clinical development and course. Some cases can be excluded when first seen as being obviously incurable, and we are not concerned here with the palliative effects of treatment, however beneficial to the patient these may be.

We have selected only those cases in whom the initial treatment has been successful to the point of causing regression of the primary (in Stages I and II cases), or of the primary and secondary (in Stages III and IV cases).

Tables XII and XIII give some idea of the subsequent clinical history of those patients whose disease is apparently controlled by the first treatment instituted. Three years have been arbitrarily chosen as the period for which success or failure of the first treatment has been calculated, because the great majority of recurrences from cancer of the mouth occur during this period.

TABLE XII.—*Results of First Planned Treatments.*

Site :—	Lip.		Mouth.	
Stage :—	I II	III IV	I II	III IV
Complete regression of disease following first treatment	90	29	256	248
No recurrences within the first 3 years	70	10	108	45
Recurrences within the first 3 years	20	19	148	203
	(22%)	(65%)	(58%)	(82%)

Many of those who recurred within the first three years were given subsequent treatment to the recurrences. The number of those cases thus re-treated who survived another 3 years is given in Table XIII. It is clearly shown that except

TABLE XIII.—*Results of Treatment to Recurrences.*

Site :—	Lip.		Mouth.	
Stage :—	I II	III IV	I II	III IV
Number of recurrences within the first 3 years	20	19	148	203
Number re-treated	20	19	71	86
Number successfully re-treated 3 years no recurrence	10	0	19	14
	(50%)		(13%)	(7%)

in Stage I and II cancers of the lip, re-treatment is rarely successful. If the first planned treatment fails, the patient does not often have a second chance.

Response of Primary and Secondary to Irradiation.

Glücksmann (1948) has stated that the response to irradiation of the primary and secondary manifestations of cancer of the mouth is about the same, and that those growths "with a tendency to lymph-node involvement differ in their biology and radio-curability from those without such a tendency." The more generally held view is that while the primary may be "cured," the secondary is often more resistant to treatment.

Only Stage III and IV cases can be analysed if the theory that an irradiated primary will recur as frequently as an irradiated secondary is to be examined.

There were 248 patients with Stage III and IV growths arising from buccal sites in whom radiotherapy produced regression of both primary and secondary growths. Forty-five remained well, and 203 recurred: 61 in the primary only, 69 in the secondary only, 73 in both. We can say then that recurrence took place in the primary 134 times and in the secondary 142 times— a difference which is only very slight. In considering the 73 cases where recurrence took place in primary and secondary, it is possible to argue that the recurrence may have occurred first in the primary, which then metastasized for the second time to lymph nodes, thus producing apparent recurrence in the secondary. If this were so there would have been 134 recurrences in the primary against only 69 in the secondary. Glücksmann's observations therefore appear to be confirmed, and it may be that recurrence in the primary is actually more common than recurrence in the secondary.

COMPARISON OF RESULTS OF TREATMENT.

It is difficult to draw conclusions from comparison of the statistics of different centres and authorities unless the material as well as the results can be compared. Owing to the lack of agreed criteria as regards the staging and position of the primary neoplasm accurate statistical comparison is impossible.

TABLE XIV.—*5-year Net Survival Rates.*

	Holt Radium Institute. 1934–38: 1250 cases.*				Royal Cancer Hospital. 1936–43: 617 cases.†				Royal Cancer Hospital 5-year survival figures adjusted to compare with Holt Radium Institute.‡
	Number of cases.	Stages I and II. %	Stages III and IV. %	5-year survival all stages. %	Number of cases.	Stages I and II. %	Stages III and IV. %	5-year survival all stages. %	%
Lip	175	84	16	65	122	76	24	67	73
Whole tongue	359	62	38	25	180	43	57	18	25
Buccal sites	407	57	43	33	220	39	61	23	27
Oro-phar' sites	309	42	58	17	95	35	65	15	17

* Tables XXXIV and XLV, 1946 Report. † Tables VI, VII, VIII and IX.

‡ Dr. H. O. Hartley has kindly done a significance test on these figures. The number of cases has been adjusted so that the proportion of Stage III and IV Royal Cancer Hospital patients is the same as that of the Holt Radium Institute series. He states that all differences are statistically insignificant.

Certainly there is no reason for complacency. Butlin's (1908) figure of 28 per cent 3-year survivals for the surgical treatment of cancer of the tongue between 1880 and 1908 (197 cases) still bears comparison with the figures of 40 years later.

The method of recording results at the Holt Radium Institute at Manchester (1946) approximates most closely to that of the Royal Cancer Hospital, and Table XIV gives an accurate comparison of results between these two centres. It will be seen at once that the Holt Radium Institute in 5 years had twice as many cases as the Royal Cancer Hospital had in 8 years. It should also be noted that they were fortunate in getting their patients at a considerably earlier clinical stage than other centres. In the case of the anterior two-thirds of the tongue, Stage III and IV cases numbered 48 per cent at the Royal Cancer Hospital, as against 38 per cent at the Holt Radium Institute. At the Westminster Hospital Cade (1949) reported almost 60 per cent with palpable nodes when first seen, and at the Radiumhemmet Jacobsson (1948) reported 48 per cent.

Table XV is an attempt to compare the published figures of five different authorities. They have been selected as offering the widest difference in methods of treatment, and the following brief summary explains how this is so:

Royal Cancer Hospital.—Roughly two-thirds of the cases of buccal and oropharyngeal cancer have been treated by teleradium and one-third by high voltage X-ray therapy. Twice as many patients with lip cancer have been treated by low voltage X-ray therapy as by radium. Surgery has seldom been used even in the treatment of cervical lymph node metastases.

Holt Radium Institute (Manchester, 1946).—Radium implants (planar and volume) were used for the treatment of the primary growth in 80 per cent of

TABLE XV.—*5-year Survivals: All Stages.*

	Royal Cancer Hospital, London. 1936–43.	Holt Radium Institute, Manchester, 1946 (Radium only). 1934–38.	Westminster Hospital, London (Cade, 1949.) 1925–37.	Memorial Hospital, New York (Martin, 1948.) 1935–42.	Stockholm Cancer Society, 1949. 1931–42.
	%	%	%	%	%
Lip	66	65	50	70	—
Tongue	18(1)	28	23	30	28
Floor of mouth	25(2)	33	42	20	30
Buccal mucosa	17	46	61†	24	35
Alveoli	27(3)	33	16†	32	28
Hard palate	20(4)	35	54†	30	—
Soft palate	13(5)	19(5)	5		—
Tonsil	19		24	20	24

(1) Except inferior surface.
(2) With inferior surface of tongue.
(3) Lower alveolus only.
(4) With upper alveolus.
(5) With anterior pillar.
† Small number of cases.

* The figures from the Royal Cancer Hospital and from the Holt Radium Institute are net survival rates. Owing to differences in methods of recording, the figures from the other centres are not strictly comparable.

buccal and faucial cases, and high voltage X-ray therapy in the other 20 per cent. Teleradium was not employed. Almost all lip cancers were treated by a radium mould or needles. Block dissection was done on mobile cervical metastases, X-rays or radium treatment given to the inoperable cases.

Westminster Hospital (Cade, 1949).—The figures in the table are taken from Sir Stanford Cade's book. All forms of radium therapy have been employed, but with the emphasis on interstitial needling for the accessible growths of the mouth and lip, and teleradium for the oro-pharyngeal primaries. For cervical metastases block dissection has been the method of choice, or radium therapy for the inoperable cases. X-rays have been used comparatively rarely.

Memorial Hospital, New York (Martin, 1948).—Dr. Hayes Martin states that surgical excision is the method of choice for cancer of the lip, gum, anterior portion of the tongue, cheek, floor of mouth or palate. For oro-pharyngeal growths a combination of roentgen therapy and interstitial radiation is indicated. Cervical metastases are treated by dissection when operable and by X-rays when not.

Radiumhemmet, Stockholm (Berven, 1937).—For more than 20 years the usual treatment for growths of the tongue and most other buccal sites has been teleradium, with diathermy coagulation of any residual mass. The neck has been treated by teleradium, followed, if necessary, by block dissection.

SUMMARY.

Eight hundred cases of cancer of the buccal cavity treated at the Royal Cancer Hospital between 1936 and 1945 are presented and analysed. These are grouped in a manner which seems to satisfy the anatomical, clinical and therapeutic aspects of the disease most conveniently.

Seventy per cent of patients were over 60, and ten times as many men were affected as women. Sixty-five per cent had symptoms for less than 6 months before treatment commenced, yet (with the exception of carcinoma of the lip) in 60 per cent of cases the disease had already metastasized to the cervical lymph nodes when first seen. The treatment of these 800 cases has been largely by radiotherapy.

Comparison of the results of treatment published by different authorities is difficult because of lack of uniformity in criteria adopted. Table XV shows wide divergencies for instance in the survivals of patients with growths of the buccal mucosa, and to a lesser extent of the alveoli and palate. This is largely due to the small numbers of the cases.

The last section of this paper shows that although very different methods of treatment may be employed, the results on the whole are much the same. It is therefore inadvisable to attempt definite conclusions from the analysis of the cases presented. The following views are therefore put forward only tentatively :

Carcinoma of the lip is best treated by superficial X-ray therapy.

Small Stage I primary growths in accessible situations within the mouth may be widely excised, but can be treated with equal success by radiotherapy.

The patient should be seen in the follow-up department every month in order that any recurrence in the primary or metastasis in the cervical lymph nodes may be speedily attacked.

Recurrence in the primary is more common than is usually appreciated, and

may be as frequent as recurrences in the nodes after both have been apparently successfully treated.

In most buccal and oro-pharyngeal sites the results of teleradium therapy are superior to those of X-ray therapy.

The teleradium treatment of growths of the anterior two-thirds of the tongue has not been so successful as the interstitial methods employed elsewhere.

This paper is based on a report prepared at the direction of the Clinical Research Sub-Committee of the Royal Cancer Hospital. The cases have been under the care of all members of the staff of the hospital, past and present, to whom grateful acknowledgment is made for permission to publish them. In the preparation of the paper much help was derived from Copeland-Chatterton punch cards, many of which had been completed by Dr. J. C. Dore, and from Hollerith cards. The latter system of record keeping was adopted by the Royal Cancer Hospital in 1945.

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ANDROGEN THERAPY IN 70 CASES OF ADVANCED MAMMARY CARCINOMA.

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LATHROP AND LOEB (1916) showed that the incidence of mammary cancers in female mice could be reduced, and their time of appearance delayed, by castration at an early age. This was the beginning of intensive experimental study of the relation between new growth and hormonal environment, but clinicians had long been aware of a connection between ovarian or testicular activity and mammary and prostatic tumours. White (1893) described the regression of uterine fibroids after castration, and two years later he and others reported the relief of symptoms due to prostatic hypertrophy by the same means (White, 1893, 1895; Hayden, 1895). Beatson (1896) described the beneficial influence of castration in two cases of advanced mammary carcinoma; his work was rapidly confirmed, and for a decade or so the method achieved popularity. For various reasons which will be discussed later the practice became obsolete, but the records contain many instances of great benefit conferred by it (Beatson, 1896; Boyd, 1900; Thomson, 1902; Morris, 1902; Lett, 1905). The method of interrupting ovarian activity by means of X-rays came into use during this period (Halberstaedter, 1905), and was applied at a few centres to cases of advanced mammary carcinoma (de Courmelles, 1909, 1926; Wintz, 1926), but more general interest in the subject has been revived only during the past ten years, during which the work of Huggins and others on prostatic carcinoma (Huggins and Hodges, 1941), the application to clinical practice of pure substances with oestrogenic or androgenic properties, and the discovery of retardation or even temporary arrest of activity of various malignant lymphomas in man by means of nitrogen mustards and urethane have opened up fresh lines of inquiry (Rhoads, 1946; Paterson, Haddow, ApThomas and Watkinson, 1946). The present widespread use of androgens in cases of advanced mammary cancer dates from their administration, accompanied by castration, by Ulrich (1939*a*), while the beneficial effect of oestrogens in the same malady was first reported by Haddow, Watkinson, Paterson and Koller (1944).

In this paper the clinical aspects of androgen therapy in 70 cases of advanced mammary cancer in women are described, the literature surveyed, and the effect of androgens compared with that of castration carried out by surgery and by X-irradiation. The prophylactic use of androgens in mammary cancer as described by Prudente (1945) is not discussed here.

The investigation was begun in December, 1947, and since then sufficient experience has been gained to permit an assessment to be made in general terms of the place of androgens in treatment. Many problems of practical importance remain unsolved, such as the uncertainty in predicting success, the best preparation to use, the most effective dose, and the prevention of undesirable side effects. The most disappointing fact which emerges from the work is that sooner or later

every case, even where no clinical evidence of disease remains, relapses in spite of continued treatment. Nevertheless, the extent and duration of remission is often sufficient to confer great benefit, and the utility of the therapy is beyond question.

The first 20 patients were treated by subcutaneous implantation of pellets of fused testosterone. Almost all improved as a result of this treatment, but it soon appeared that this improvement was unrelated to the progress of the disease, which often advanced unchecked. It consisted in an increased sense of well-being, improved appetite, weight gain, rise in haemoglobin value ; in other words it represented the benefit which the layman expects but rarely derives from a "tonic." Such non-specific benefit generally occurs whenever testosterone is given, and has unfortunately been used to justify testosterone therapy for other specific maladies as well as for mammary carcinoma. In the present series of cases a high percentage of the cases derived such benefit, but this has been ignored in assessing the results.

In only 6 of the first 20 patients was any specific benefit conferred, and it was decided that implantation as an initial method of administration was impracticable. However, when specific improvement did occur, it was already manifest within 2 months of implantation, and it therefore seemed desirable to defer implantation until the response to the hormone given by some other route had been ascertained. Implantation could then be considered in cases responding favourably, non-specific benefit being neglected. Two preparations were available, namely intramuscular testosterone propionate in oily solution, and methyltestosterone for sublingual absorption. The former has been widely used during the past 10 years for mammary carcinoma, but there are no published accounts of the large scale use of methyltestosterone, and it was decided to try this compound in subsequent cases. The early results were surprisingly good, and warranted extended trials of the substance. Most of the later cases were treated with methyl-testosterone. In a few instances testosterone implantation, sublingual methyltestosterone, and testosterone propionate injections were used in the same patient at different times, but not in sufficient numbers to permit a direct comparison of the methods. Lastly, testosterone propionate incorporated in an ointment was applied locally in a few cases of malignant ulceration of the skin.

PREPARATIONS, ROUTES OF ADMINISTRATION AND DOSAGE.

The number of patients treated by testosterone, testosterone propionate, and by methyltestosterone, is set out in Table I. These three substances differ

TABLE I.—*Response to Different Androgen Preparations.*

Preparation.	Success.	Moderate success.	Failure.	Total.
Testosterone (implant.)	5 (3)	7 (5)	14 (6)	26
Methyltestosterone (sublingual)	12 (5)	8 (2)	20 (7)	40
Testosterone propionate (intramuscular)	2 (1)	4 (3)	3 (1)	9
Combined—				
Test. and Meth.	2 (1)	1 (1)	..	3
Test. and T. prop.	0	0	0	0
Meth. and T. prop.	1	3 (2)	2	6
All three	1 (1)	0	0	1

Figures in brackets indicate cases with skeletal involvement. Cases treated concurrently with X-rays omitted.

considerably in their pharmacological properties. The androgenic potency of a compound can be expressed only in relation to the method of assay; for example, a given compound may be more potent than another in stimulating the growth of the seminal vesicles of immature rats, but less so when the weight of the prostate is measured for assay. In the main these differences probably reflect varying speeds of absorption, cumulation and excretion rather than inherent pharmacological differences. However, testosterone is usually stated to be a slightly less potent androgen than testosterone propionate, but more powerful than methyltestosterone.

Testosterone.

Subcutaneous implants in the form of 50 mg. and 100 mg. pellets of fused amorphous testosterone prepared from cholesterol (Butenandt and Hanisch, 1935) were used. In 18 cases 500 mg. was given, in 11 cases 1000 mg., 3 having received a previous implant of 500 mg. The pellets were inserted through a skin incision 1 cm. long in the lower abdominal quadrants, and pushed through radially to rest on the external oblique aponeurosis between 5 and 10 cm. from the incision, the pellets lying at points on a circle with the incision as centre.

Absorption from these pellets depends on a variety of local factors, including their relation to surrounding fat, adjacent fascia, lymphatic plexuses and encapsulation by fibrous tissue. In three patients who came to autopsy 55, 82 and 93 days respectively after insertion, the pellets were removed, allowed to dry for 48 hours and weighed. The weights of these pellets are set out in Table II, from which it is seen that absorption rate is not uniform. The smooth, thin, translucent fibrous tissue capsules in which the pellets were embedded were well established in the case in which implantation was carried out 55 days previously, and were no thicker as judged by naked-eye inspection in the case which came to autopsy 93 days after implantation.

TABLE II.—*Dry Weights of 100 mg. Testosterone Pellets Removed at Intervals after Implantation.*

	Case 48.*	Case 11.	Case 65.
Days since implantation	55	82	93
Dry weight in mg. of recovered pellets	35·6	44·7	40·8
	34·2	51·0	31·9
	29·3	53·5	26·4
	24·8	53·0	23·7
		51·0	13·1
Total unabsorbed testosterone (mg.)	154·8	253·2	135·9
Total absorption (mg.)	345·2	246·8	364·1
Mean absorption per pellet (mg.)	69·0	49·4	72·8
Greatest absorption	75.2	55.3	86·9
Least absorption	64·4	46·5	58·2
Variation	10·8	8·8	28·7

* One pellet was not recovered from this case. In calculating total absorption the weight of the missing pellet was taken as the mean of the weights of the other 4.

Absorption from implanted pellets is maximal at the time of insertion, and then diminishes at a rate which is proportional to the rate of decrease of the

surface area of the pellets. With ten 100 mg. pellets the maximal daily absorption probably never exceeds 10 mg.; nevertheless the constitutional effects produced by this dose require about 500 mg. per month for their evocation when injections of testosterone propionate are used (Geist, Salmon and Gaines, 1938; Geist, Salmon and Walter, 1940), representing daily doses of nearly double the maximal (initial) daily absorption from ten 100 mg. pellets. It seems likely, therefore, that the injection of testosterone propionate is attended by considerable wastage, and that the much smaller quantity of hormone utilized by continuous slow absorption from implanted pellets more nearly represents the pharmacologically effective amount. This view has received experimental confirmation. Carlinfanti, D'Alo and Cutolo (1949) showed that the activity of a single dose of testosterone in preventing post-castration atrophy of the seminal vesicles of guinea-pigs could be greatly prolonged and also enhanced by preliminary adsorption on to aluminium phosphate. Absorption from the suspended particles may be compared to that from multiple small implants.

The observations on which the above conclusions are bases are admittedly few, and the variable absorption from implanted pellets, and the lack of control and of precise knowledge of absorption once implantation has been carried out, may be considered to limit the reliability of the method. But in practice it has yielded results of value, and against its disadvantages may also be set the facts that patients receiving injection therapy require repeated visits by district nurses, as few patients can be taught to give their own intramuscular injections, while sublingual therapy is open to the very real objection that administration is unsupervised and therefore less certain. Further, the question of expense cannot be ignored, and at present the method of implantation is the most economical way of giving androgens provided it is confined to cases already known to respond to androgen.

In retrospect the dose of 500 mg. by implantation which was given 18 times appears insufficient, but implantation of 1000 mg. (10 × 100 mg.) is probably sufficient to secure specific response in mammary carcinoma. It is recommended as the method of choice for maintenance therapy in cases which have shown a satisfactory response to intramuscular or oral therapy, but should not be used without a preliminary trial, owing to the high percentage (80 per cent) of cases which fails to respond to androgen therapy in any form. No instance of pellet extrusion occurred.

Testosterone propionate.

This substance in oily solution for intramuscular injection has been the most widely used androgen preparation in cases of mammary carcinoma. In the earlier reports doses of the order of 25–50 mg. were given 3–7 times weekly, but later opinion came to advocate larger and larger doses of the order of 100 mg. daily, or even twice daily. The authors of the latest progress report of the Council on Pharmacy and Chemistry (1949), however, no longer favour heavy dosage, and consider that there is no advantage in exceeding 150 mg. weekly, this view being based on the results obtained in 285 cases in which dosage schedules between 25 mg. and 200 mg. thrice weekly were employed.

In the present series only 9 cases have received testosterone propionate, in all but two instances it was used after methyltestosterone had failed to initiate or maintain a remission, and in only 2 of the 9 cases was it effective in any degree.

In every case the dose given was 100 mg. daily for the first fortnight; one case received no further treatment, 4 others continued daily injections for another month, the remaining 4 continuing on 3 injections weekly for 6–10 weeks.

17-*Methyltestosterone.*

This compound is more readily absorbed from mucous membranes than either the pure hormone or its esters, but is a more feeble androgen. Judged by its effect on menstruation, on the skin, hair and general metabolism, it is about 1/5 as active weight for weight as testosterone, and its administration is even more wasteful than that of testosterone propionate. However, in this country it is less expensive in the doses here recommended than the propionate, but more so than testosterone implantation. Of the 44 patients who were given methyltestosterone, 28 received 50 mg. daily for indefinite periods between 3 months and 1 year; the remainder received larger doses, 100 mg. daily being the largest given. The present tendency is to give 100 mg. daily for the first 2–4 weeks, to halve this dose if the patient is responding favourably, or to continue for another month if there is no evidence of specific benefit. All 30 patients who derived benefit from androgen therapy showed clear evidence of it within 8 weeks, and in most cases within the first month. No patient who failed to respond after 8 weeks of treatment did so after a longer interval, and it is probably not worth continuing treatment after 10 weeks in the absence of specific response. It cannot be said from the small number of cases in this series whether such cases should be transferred to testosterone propionate injections, but the general impression gained has been that patients likely to benefit substantially from androgen therapy are extremely sensitive to its action, in whichever form it is given, and do as well on doses just large enough to suppress menstruation in the average premenopausal woman as they do on much larger doses. On the other hand, most cases which fail to respond to the smaller doses do no better when the dose is increased or the route of administration changed, but the minority which does so led to the tendency already mentioned to advocate heavy dosage.

Patients given methyltestosterone were advised to leave the tablets (50 mg.) under the tongue, and to allow them to dissolve without chewing or swallowing. The time taken for solution varied between 10 and 30 minutes in different individuals, and a few patients disliked the bitter taste of the substance.

Duration of Treatment in Cases Responding Favourably.

No definite recommendation can be given with regard to the length of time administration should be continued. Cutler and Schlemenson, reporting 19 cases, advised continuing injections until palliation was no longer being obtained (Cutler and Schlemenson, 1948). The American Council already referred to found that the most favourable results were obtained when injections were continued until a total of 3·0 g. had been given. In this series it has frequently been observed that lesions which heal during treatment do not later break down when recurrences appear at other sites, whether treatment is still in progress or not. Also, in 2 cases (25 and 52) no treatment other than a single implantation of 1000 mg. and 500 mg. respectively was given. In Case 25, malignant pleural effusions and pulmonary deposits having resolved, the patient is recurrence-free 14 months later, while in Case 52 there has been no recurrence of back pain and

no clinical or radiological evidence of extension of the disease 18 months later. On the other hand, a majority of cases relapsed in spite of continued treatment. In a few instances the disease process even appeared to be accelerated by continued treatment. In Case 37, reported more fully in the Appendix, dramatic improvement followed implantation of 500 mg. (10 × 50 mg.) testosterone, was maintained by a second implantation of 1000 mg. (10 × 100 mg.) four months later, and by 50 mg. methyltestosterone daily, commenced 6 months after the second implantation and continued for a further 4 months. Widespread evidence of fresh activity of the carcinoma then appeared, accompanied by recurrence of pain and general deterioration. Testosterone propionate injections 100 mg. daily were then given over a period of 6 weeks without any benefit, after which androgen therapy was discontinued. Since then the patient's general condition has again improved, and regression of cutaneous, skeletal and lymph node metastases has occurred, the original implantation having been carried out 20 months ago. Adair (1949) recorded a case in which treatment was continued for many months after initial improvement in spite of deterioration. Cessation of treatment was almost immediately followed by striking and maintained remission. These facts suggest that there is a limit to the amount of testosterone which can be usefully given, beyond which further administration is unlikely to be of value. If a lesion has regressed it will not recur. If it has only partially regressed it will resume its activity whether more hormone is given or not. In a patient with healed lesions, more testosterone will not prevent recurrence at other sites. Further observations are required.

THE SIDE EFFECTS OF ANDROGEN THERAPY.

These include the beneficial effects which have been referred to earlier, as well as the less desirable masculinizing effects and other disturbances. Evidence will be given later indicating that the masculinization which is a common accompaniment of testosterone therapy is not a necessary condition for success in the treatment of mammary carcinoma. It is therefore included as a "side-effect." The chief side-effects may be grouped as (1) oestrogen withdrawal effects, (2) signs of masculinization, and (3) metabolic effects.

Oestrogen withdrawal effects.—These are observed within the first month of treatment, and consist of hot flushes, regression of the endometrium with amenorrhoea, and conversion of the vaginal epithelium to the "oestrin-deficient" stage (Papanicolaou, Ripley and Shorr, 1938). They are met with in cases of surgical castration, and somewhat later in cases of X-ray induced castration.

Signs of masculinization.—These become prominent during the second 3 months of treatment, and consist in order of appearance in coarsening and increased sebaceous activity of the skin, facial hirsuties, acne, increased growth of hair on the limbs, huskiness of the voice and enlargement of the clitoris (Geist, Salmon and Walter, 1940; Zuckerman, 1937; Foss, 1938).

Metabolic effects.—Several symptoms and signs are to be ascribed to metabolic changes, which include re-mineralization of the bones (Farrow and Woodard, 1942), retention of water, electrolytes and nitrogen (Kenyon, Sandiford, Hughes, Knowlton and Koch, 1938; Abels, Nelson, Young and Taylor, 1944), and others less easy to define. The patients become alert and active, and gain strength and weight. They may complain of swelling of the ankles. When hypercalcaemia occurs the patients complain of drowsiness, thirst, headache, nausea and vomiting.

In one patient with hypertension, the onset of angina of effort which seemed to be precipitated by testosterone therapy was probably due to the increased activity and weight gain increasing the load of a heart already failing (Case 16). This may have been the precipitating cause in 5 of 24 cases reported by Taylor, Slaughter, Smejkal, Fowler and Preston (1948), in which congestive cardiac failure followed androgen therapy.

These phenomena provide a rough measure by which the absorption and activity of the different preparations may be compared. Thus implantation of 500 mg. testosterone was followed by amenorrhoea for 4 months in both patients in whom it was carried out. Implantation of 1000 mg. led to amenorrhoea lasting 7 months. With ten 100 mg. pellets the maximal absorption of testosterone is probably less than 10 mg. daily. By contrast daily administration of 50 mg. methyltestosterone sublingually caused amenorrhoea in 7 out of 11 women, menstruation being unaffected in 1, reduced in another, and disturbed in the remaining 2. Forty mg. daily was without effect on menstruation. Doses over 80 mg. induced amenorrhoea. As far as the effect on menstruation is concerned, it may therefore be said that the hormone absorbed from 50 mg. sublingual methyltestosterone is less potent than 10 mg. of testosterone subcutaneously. With varied doses it has also been possible to compare the thresholds for the appearance of different side-effects. Thus patients who received 500 mg. testosterone implants almost all gained weight in the first month, in one case as much as 8 lb., but skin changes were never marked with this dose, facial hirsuties developed in only 2 out of 18 cases, and voice change was marked in only 1 case. Of the 3 fatal cases from which the pellets were recovered, one gained 7 lb. in weight during the first month following implantation, and at the time of death 9 weeks later had still absorbed only 364·1 mg. testosterone (Table II). Marked facial hirsuties was present in the second of these cases at the time of death, 12 weeks after implantation, not more than 250 mg. testosterone having been absorbed. These changes occurred much more often in patients who received 1000 mg. implants, and in those who took sublingual methyltestosterone in doses over 50 mg. daily. In the case of implantations the effective dose diminishes every day, but even with 500 mg. amenorrhoea persisted for 4 months, and weight gain continued during the same period. Weight gain, and subjective benefit, occurred without other side-effects in patients who were given methyltestosterone in 40 mg. doses daily. It therefore appears that the production of masculinizing effects requires larger and more prolonged dosage than that necessary to initiate the metabolic changes responsible for increased activity, appetite, sense of well-being, and weight gain ; inhibition of menstruation requires intermediate dosage. The order of appearance of these effects in the individual patient runs parallel to their incidence in groups receiving different doses.

The " Tonic " Effect of Testosterone.

The speedy improvement brought about by testosterone therapy even in debilitated patients is very striking. Weight gains of 5 lb. in the first month are common, and with the other " tonic " effects are independent of the specific effect of the hormone on mammary carcinoma. In Case 65 for example 7 lb. weight gain occurred in the first month, during which rapid extension of the disease in chest wall, regional lymph nodes and opposite breast occurred. Weight gain may

reach embarrassing levels. One patient (Case 35) gained steadily at the rate of 3 lb. per week, and in 3 months had put on 32 lb. Another (Case 44, Appendix) gained 23 lb. in the first 4 months, and a further 8 lb. in the second 4 months. Clinical oedema, however, was seen in only 3 cases, and was always slight in amount, and confined to the ankles and lower half of the legs. In a few of the patients with post-mastectomy oedema of the arm, an increase in the swelling of the arm followed testosterone administration, but this was more closely related to spread of the disease than to the treatment, for when lesions regressed, the lymphoedema sometimes diminished, even when rapid increase in weight was occurring, as in Case 44. During the initial period of weight gain, some of the patients stated that they passed less water, but no measurements have been carried out.

Much of the initial rapid weight gain is due to the effects of electrolyte, and particularly sodium retention, which holds equivalent quantities of water in the body. This effect is a feature of the activity of many steroid hormones, notably desoxycorticosterone, progesterone, oestradiol, and of Kendall's compounds E and F. When administration of the hormone is discontinued, much of the rapidly gained weight is lost, and if it is continued the rate of weight gain diminishes and finally comes to a standstill. Part of the weight gain is due to increased appetite, and in sick patients who obtain specific benefit, improvement in general health also contributes, and extra tissue, especially fat, is laid down.

Rise in haemoglobin occurs during the first few months of treatment, and may be considerable, even when widespread skeletal metastases are present.

The Effect of Testosterone and Methyltestosterone on Menstruation.

The number of menstruating women in this series is small, and the results insufficient to permit a full account of the inhibiting action of these androgens. The effect of testosterone propionate has been fully studied by others (Papani-

TABLE III.—*Effect of Testosterone and Methyltestosterone on Menstruation.*

Day of menstrual cycle treatment commenced.	Age (years).	Preparation and dose.	Next menstrual period.
3	43	Methyltestosterone 50 mg./d.	Absent.
3	48	Methyltestosterone 50 mg./d.	„
3	37	Methyltestosterone 50 mg./d.	Slight "staining" for subsequent 16 days. Next period absent.
4	37	Testosterone implant. 1000 mg.	Absent.
5	42	Methyltestosterone 100 mg./d.	„
7	40	Methyltestosterone 50 mg./d.	Next period 8 days early (14 days after). Following period also 8 days early.
9	43	Methyltestosterone 80mg./d.	Reduced in amount but punctual. Following period absent.
10	37	Testosterone implant. 500 mg.	Absent.
11	37	Methyltestosterone 50 mg./d.	Next period 8 days early (11 days later). Following period absent.
16	48	Methyltestosterone 50 mg./d.	Absent.
26	39	Methyltestosterone 50 mg./d.	Slight " staining " for ½ day on 1st day of next 2 periods.

colaou, Ripley and Shorr, 1938 ; Geist, Salmon and Walter, 1940). In 11 women with regular periods the day on which testosterone therapy was begun was noted, counting the first day of the last menstrual period as day 1. The effect on the next expected menstrual period is indicated in Table III. The next period may be suppressed, reduced in amount of bleeding and duration, or may be brought on about a week early. In one of the cases where methyltestosterone was given during a menstrual period (on day 3), slight bleeding continued until the 19th day of the cycle, the next period being suppressed. Amenorrhoea persists as long as administration of the hormone is continued at the necessary dosage, the periods returning regularly about a month after withdrawal. One patient (Case 37) in whom amenorrhoea lasted for a year had a single " period " a fortnight after treatment was finally discontinued, but has not bled since.

The Masculinizing Effects of Androgens.

The skin and voice changes which almost always follow continued administration of androgens are both unpleasant and embarrassing for the patients, but are usually tolerated without complaint when much specific improvement occurs. These changes usually became marked during the third month of treatment, but much variation in their intensity was observed, and a few patients escaped some or all of them. Routine examination of the genitals was not carried out, but in a few patients hypertrophy of the clitoris was noted, involving the shaft, glans and hood, under which much smegma had accumulated.

Skin changes.

The primary skin changes are increased activity of the sebaceous glands (Roug and Zakon, 1943), of the hair follicles, and increased keratinization, more particularly at the mouths of the lanugo follicles. The seborrhoea is often severe, especially in dark complexioned subjects, and on the scalp pityriasis capitis may develop. The texture of the skin becomes coarser, the follicles become more prominent and sooner or later some become infected, and a punctate folliculitis ensues, being most marked in the interscapular and intermammary regions. On the forehead and chin the lesions tend to be scantier but larger, and the patients complain of recurrent crops of " pimples." The general coarsening of the skin, with prominent " pores," especially on the forehead, cheeks, chin and sides of the nose sometimes alters the facial appearance of the patient sufficiently to excite the comment of relatives or friends. The skin condition resembles pustular acne, but differs from it in that comedones rarely develop, even when the lesions persist for many months. In two patients only (Cases 63 and 44) did true comedones appear after taking methyltestosterone, 50 mg. daily for 6 months and 1 year respectively. They were abundant across the back, and were also present on the cheeks and chin. The skin, which was heavily infected, had the lumpy appearance typical of adolescent pustular acne. In Case 44 hirsuties was minimal, consisting of a sparse growth of soft hairs on either side of the upper lip, but was much more developed in Case 63. In 7 cases facial hirsuties reached an extreme degree, the sides of the face being covered with thick downy hair between 1 and 2 cm. long (Cases 7, 9, 24, 25, 56, 63). In Cases 9 and 56, after 6 months' treatment the hairs increased in calibre, and resembled the stiff bristles of the male beard. There was some variation in the changes in the scalp hair. Some patients maintained that their hair grew more vigorously, and more thickly, while

others averred that their hair " fell out in handfuls." In two cases (25 and 63) marked hair recession in the temporal region was observed. Increased growth of body hair was less commonly seen, but was marked in two cases, both with dark hair (46 and 56), the shoulders, abdomen, forearms, thighs and legs being heavily affected. Apart from these two cases, upward spread of pubic hair towards the umbilicus was not met with.

Voice changes.

Voice changes occurred only in a proportion of patients with relatively high-pitched voices. In only 12 was definite lowering of pitch recorded, but lesser degrees of change were noted in several others. The first evidence of change consists in slight huskiness, which may become obvious only after the voice has been used for some time. The condition may then remain stationary, or may progress to more permanent huskiness, and in a smaller number of patients lowering of pitch with increased resonance ensues.

Duration of effects.

The persistence of the skin changes is dependent on a continued supply of androgen, and the lesions clear up within two months of withholding the supply of methyltestosterone. The hirsuties remains longer, but rarely persists 6 months after stopping treatment. The voice changes are more permanent, and may last for a year or more. It is difficult to explain the mechanism of the voice change, but its persistence suggests some structural alteration in the larynx. Radiographs have not revealed any changes in calcification of the laryngeal cartilages. In two instances (Cases 43 and 63) the skin condition was most advanced at the end of the third month of treatment, and then regressed considerably in spite of continued treatment with methyltestosterone.

Toxic Effects.

The side effects that have been described above are at most inconvenient, but in themselves are not harmful. On occasion, however, androgen administration can precipitate toxic symptoms of some severity. When fully developed they form a characteristic syndrome, which has been observed in 3 patients in this series. Lesser degrees of it were met with in 7 others. It was first seen when 2 patients (Cases 10 and 45) were given 80 mg. methyltestosterone daily in 5-mg. tablets, both patients having been treated 5 months previously by implantation of 500 mg. testosterone. In Case 10, after taking the tablets for 2 days, the patient complained of extreme weakness, drowsiness, severe headache, nausea, and increase in the dyspnoea which was her initial complaint, and due to pulmonary and pleural metastases. The symptoms subsided rapidly after she stopped taking the tablets. In Case 45 the patient took the tablets for 5 days, during the last 3 of which she felt generally ill, nauseated, and complained of increase in the severity of her backache. On the 5th day she was distressed by vomiting, abdominal distension and pain, and took no more tablets, after which her condition improved rapidly. One week later she again attempted to take the tablets, but with exactly the same result. Case 30 was a woman of 45 who had been previously treated for 2 years by repeated doses of X-rays for widespread skeletal recurrences for a Stage IV carcinoma, and had had induction of an

artificial menopause. She was given methyltestosterone 50 mg. daily for one month, towards the end of which she complained of increase in the severity of her pains, dizziness, nausea and vomiting, and general malaise. The symptoms abated within a day or two of discontinuing the drug. She was asked to resume the tablets a fortnight later, but the symptoms recurred after one week. In Case 47, doses of 35 mg. methyltestosterone regularly induced anorexia, extreme thirst, dizziness, headache, fever, nausea and vomiting, these symptoms decreasing rapidly when the substance was withheld. In the mildest cases drowsiness or depression were the only symptoms, and cases intermediate in severity occurred also. This syndrome has been ascribed to the flooding of the circulation with calcium ions mobilized from the bones, and is said to be precipitated more readily by testosterone when the serum calcium level is initially high (Farrow and Woodard, 1942). Similar symptoms occur when large doses of calciferol are administered over a long period, and are also associated with elevation of serum diffusible calcium level (Anning, Dawson, Dolby and Ingram, 1948). The present cases have not provided additional information on this point.

Gastric symptoms.

Five patients (Cases 4, 7, 15, 52, 70) developed gastric symptoms during testosterone therapy. Three of them (Cases 4, 52, 70) had a previous history of peptic ulceration, and in all three investigations indicated active ulceration. In one of these patients (Case 52) the testosterone was implanted, but the other 4 took sublingual methyltestosterone. The two patients with no previous ulcer history complained of heartburn, acid regurgitation and eructations coming on about 30 minutes after each meal, lasting for another half hour unless relieved by alkalis. The symptoms were relieved by a few weeks on a milk diet, the testosterone being continued.

Cardiac symptoms.

Taylor, Slaughter, Smejkal, Fowler and Preston (1948) refer to the onset of tachycardia, tremor and congestive cardiac failure in their report on hormone therapy. In the present series only one case (Case 16) showed any evidence of an adverse effect on the heart, and as already suggested, this was in all probability only a secondary effect due to rapid weight gain.

Relation of Side-effects to Specific Effect.

The specific effect of testosterone is not directly dependent on any of the side-effects described. Some of the best results were obtained in patients who did not develop marked signs of masculinization, while in Case 20 regression of the disease had begun with a dose of methyltestosterone which did not suppress menstruation (40 mg. daily). On the other hand several of the patients who gained weight most rapidly, and later developed gross signs of masculinization, never obtained the slightest specific benefit, and in some the disease progressed rapidly.

Clinical Material.

All the patients referred for androgen therapy were in an advanced stage of the disease. Fifteen were Stage IV cases when first seen, 35 cases had severe symptoms, mostly generalized skeletal pain or dyspnoea, and 44 cases were found

at follow-up to have recurrences at various sites unsuitable for excision, or unlikely to respond to further irradiation. An impression of the composition of the series may be gained by the classification into 3 groups based on a general clinical assessment at the start of androgen therapy.

Group I : Good general condition.—In the first group are 40 patients in good general condition, with no recent weight loss, no anaemia, afebrile, and who would describe themselves as fit except for the particular symptom or sign requiring treatment. Thus 4 cases whose only complaint was dyspnoea are included in this group. Patients with limited disability due to bony metastases are also placed in it, for example Case 4 with a painful right shoulder, Case 70 with stiff neck, and 3 patients otherwise fit, but bedridden because of severe localized backache, with paraplegia in one case (Cases 7 and 60). Most of the patients in whom skin recurrences predominated, whether ulcerated or otherwise, also fall into this group.

Group II : Poor general condition.—The second group is composed of 20 cases in poor condition at the commencement of therapy, deteriorating rapidly with regard to appetite, weight, anaemia and general strength. Seven patients in this group were bedridden, and all the others were leading a sedentary existence and unable to look after themselves. Ten had widespread skeletal metastases with severe pain.

Group III : Fair condition.—The remaining 10 patients were intermediate in general health between the two groups just described ; all but one were ambulant, though complaining of varying degrees of weakness, anorexia, weight loss and malaise. The bedridden patient in this group had cerebral metastases causing headache, vomiting and ataxia (Case 53). In the whole series 31 patients had multiple bone deposits, 12 had intrathoracic recurrence, 30 had lymph-node involvement, 28 more or less extensive skin recurrences. Bone deposits were found radiologically in 6 patients who had not complained at any time of pain, and were probably present in others who were not X-rayed.

Age composition.

The age distribution is shown in Table I. Half of the patients were below 50 years, and 2 patients between 50 and 54 were still menstruating at the commencement of treatment. The youngest patient was 35 years, but 12 others were less than 40 years of age.

Twenty-two patients were premenopausal, 11 had had previous induction of artificial menopause, 37 had ceased to menstruate more than 1 year before commencing androgen therapy.

Recurrent cases. Time since radical surgery.

It has been suggested that a relation exists between response to androgen therapy and the interval since radical surgery. In this small series it has not been possible to isolate this relation from the age factor and from the menopausal factor, but some of the figures are given (Table I).

Fifteen cases presented with recurrence within 1 year of radical surgery. All but 2 of these were below 50 years of age, and only 3 responded favourably to androgen therapy, the disease progressing rapidly in the remainder, with widespread blood-borne dissemination in most instances.

Twenty-nine cases were alive more than 3 years after radical surgery, 13 between 3 and 5 years, 12 between 5 and 10 years, and 4 over 10 years. In 6 of the 14 cases with the best response to testosterone therapy the disease had recurred more than 3 years after radical surgery, in 7 others under 3 years after, in 1, 10 years after surgery.

Parity.

Thirty-four patients were childless, 13 of whom were married before the onset of the menopause. In 5 patients the parity was not recorded.

Classification of Results.

In Table IV the results of treatment have been set out in 3 groups, namely "success," "moderate success" and "failure." By a "success" is meant a case in which relief of specific symptoms was accompanied by objective evidence of improvement and restoration of function, the patient resuming her normal activities, or in which conspicuous and lasting regression of lesions occurred. Eight of the 14 cases scored as "successes" remained well for over 1 year, 4 are still in remission more than 1 year after commencing treatment, the disease being again active in the other 4. Three others relapsed between 6 and 12 months after commencing treatment, and the remaining 3 are still in remission 3–6 months later. By a "moderate success" is meant a case in which symptomatic relief was short-lived (mostly less than 6 months), or not accompanied by considerable objective evidence of regression or by restoration of function. All other cases are classed as failures, even when non-specific benefit was marked and increased the patients' range of activity. Among the failures are included a few patients in whom follow-up was for one reason or another inadequate, and 11 in whom treatment had been abandoned in less than 2 months.

TABLE IV.—*Response to Androgen Therapy in Relation to Menopause and to General Clinical Condition before Treatment.*

	I. Good condition.				II. Poor condition.				III. Fair condition.				Total.
	Relation to menopause.			Total.	Relation to menopause.			Total.	Relation to menopause.			Total.	
	Pre-	Artificial menopause.	Post-(natural).		Pre-	Artificial menopause.	Post (natural).		Pre-	Artificial menopause.	Post-(natural).		
Success	3	2	2	7	3	0	2	5	2	0	0	2	14
Moderate success	3	2	4	9	0	0	3	3	1	0	3	4	16
Failure	2	4	14	20	2	2	7	11	2	1	1	4	35
Radiotherapy as well	4	0	0	4	0	0	1	1	0	0	0	0	5
Total	12	8	20	40	5	2	13	20	5	1	4	10	70

Duration of Observations.

It will be seen from Table V that of the 59 patients followed up for more than 3 months, 38 have been observed for 6 months or longer, of whom 16 were observed for over 1 year after first receiving androgen therapy. Three of the 5 patients observed for over 18 months are classified as "successes." One of them (Case 37) is of great interest and is more fully described below. The other two patients observed for more than 18 months (Cases 18 and 28), one aged 68, and one aged

TABLE V.—*Duration of Observations* and Length of Remission.*

Months.	3–6.	6–12.	12–18.	18+.	Total.
Total treated	21	22	11	5	59
Failure	12	11	4	2	29
Total remissions	9	11	7	3	30
Still in remission	3	3	2	2	10
Relapsed	6	8	5	1	20

* Excluding 6 patients observed less than 1 month (classed as failures in Tables III and IV) and 5 patients treated concurrently with X-rays.

71, are both classified as failures. They were transferred to oestrogen therapy after 6 months' trial of androgens, and both responded well.

Of the 30 patients who derived benefit from the treatment, 21 obtained remissions lasting 6 months or more, 10 of them over 1 year, and 3 over 18 months. Twenty patients have relapsed between 3 and 18 months later, but 10 are still in remission at the time of writing, 4 of them 12 months or more after institution of therapy. By a relapse is meant any evidence of renewed activity of the disease, whether or not accompanied by symptoms. Case 37 has been classed as a relapse, although she has had a second remission since androgen therapy has been withheld. Cases 35 and 44 are also classed as "relapses" although both are in normal health, the former on account of a lymph node found at follow-up, and the latter because of two small recurrences in an otherwise healed chest wall ulcer.

General Survey of Results.

It will be seen from Tables IV and VI that 30 of the 70 cases showed some specific response to androgen therapy, although only 14 of these have been classed as "successes." Among the 22 pre-menopausal cases were 8 successes, whereas there were only 4 successes among the 37 patients who had passed the menopause. Of 11 cases who were given androgens after the induction of artificial menopause, only 2 could be counted as successes. Of the 15 Stage IV

TABLE VI.—*Distribution of "Successes" in Relation to Age, Menopause, and Time since Radical Surgery.*

Age group.		Relation to menopause.			Stage IV cases.	Cases with recurrence. Time from radical surgery.				
		Pre-	Artificial menopause.	Post-(natural).		1 year and under.	1–3.	3–5.	5–10.	Over 10.
30–4	0	0	0	0	0	0	0	0	0	0
35–9	13 (4)	8 (3)	4 (1)	1	2 (1)	6 (1)	3	2 (2)	0	0
40–4	8 (2)	6 (1)	1 (1)	1	0	2 (1)	4 (1)	2	0	0
45–9	15 (5)	6 (5)	4	5 (1)	4 (1)	5 (1)	2	1 (1)	3 (3)	0
50–4	18 (1)	2	2	14 (1)	5	2	1	5	3	2 (1)
55–9	8 (1)	0	0	8 (1)	3	0	1 (1)	1	2	1
60–4	3 (1)	0	0	3 (1)	0	0	1 (1)	1	0	1
65–9	3	0	0	3	0	0	1	1	1	0
70–4	2	0	0	2	1	1	0	0	0	0
75–80	0	0	0	0	0	0	0	0	0	0
Total	70	22	11	37	15	16	13	13	9	4
"Successes"	14	9	2	4	2	3	3	3	3	1

Figures in brackets indicate the number of "successes."

cases previously untreated, only one (Case 20) responded successfully to androgen therapy, the only other success in this group having had an artificially-induced menopause.

Of the 30 patients who obtained specific benefit from androgens, 16 were cases with predominantly skeletal involvement, 7 of which (4 of them pre-menopausal) have been classed as successes.

From Table IV it is seen that specific response to therapy is independent of the general condition of the patient at the commencement of therapy. Some of the most dramatic effects of androgens have occurred in patients grossly debilitated and bedridden. Thus there were 7 successes among the 35 patients in Group I (good general condition), 5 among the 19 in Group II (poor general condition), and 2 among the 10 patients in the intermediate group.

Cases with symptoms of skeletal involvement.

As others have noted, the most gratifying results of androgen therapy have been in cases with skeletal deposits giving much pain. The relief of pain often came within a few days of commencing treatment, even when the pain had been present for many months beforehand, the patients crippled and requiring repeated doses of opiates. Sixteen out of 28 cases with skeletal pain obtained some relief of symptoms, but in 7 of these the effect can only be described as spectacular. Three bedridden patients were able to walk within a fortnight of commencing treatment, and were pain-free within a month, and able to return to their normal activities. Case 37 is described in detail below; she remained in good health for 15 months, after which the pains recurred. The other 2 cases are symptom-free 14 months and 6 months after commencing treatment with testosterone. Two other patients severely handicapped by skeletal pain, though not bedridden, were able to resume their normal activities after receiving testosterone, and are symptom-free 8 and 18 months later. One, Case 4, obtained rapid initial relief of pain in the shoulder associated with a large osteolytic metastasis at the medial end of the clavicle after taking 50 mg. methyltestosterone daily. Five months later her condition deteriorated; 6 months later she developed gross mediastinal obstruction. The shoulder pain has not recurred. She was transferred after 5 months to injections of 100 mg. testosterone propionate three times weekly without benefit after 6 weeks. She is classed as a "moderate success" in view of the initial response. The remaining patients obtained less benefit, and 3 were never able to leave their beds, although all volunteered that their pains were improved. One of these declared that she had not felt so well nor had so little pain for 2 years, 20 months of which had been spent in bed.

Case 66 was a woman of 44 years with extensive metastasis to the spine which was responsible for gross collapse of the bodies of dorsal vertebrae 7–11. She had been unable to walk or stand for 6 weeks owing to severe proprioceptive loss in both legs, a short course of radiotherapy having conferred no benefit. A mild paraplegia was present, and there was some impairment of sensation to light touch, deep pressure and pinprick from the level of the umbilicus downwards. There was, in addition, a girdle of almost complete anaesthesia to touch and pinprick over the distribution of dorsal segments 7–10. Curiously, sensation over the paravertebral regions of these segments was normal. There was no hyperalgesic zone, and no disturbance of sphincter control. The patient was given 28 daily injections of 100 mg. testosterone propionate intramuscularly, followed

by 3 weekly injections for 2 weeks, and a further month of 2 injections weekly. During this period considerable improvement in proprioception occurred, the signs of paraplegia became less, though both plantar responses remained equivocal and right ankle clonus persisted, while perception of touch and pinprick returned to normal. She was able to stand with moderate rombergism, and after 3 months could walk 100 yards with one assistant. Whether this improvement reflects any specific effect of the hormone, or not, cannot be said, but it began within a month of starting treatment, whereas her condition was stationary during the preceding 6 weeks. After hesitation it has been decided to include her as a "moderate success."

Cases with symptoms of intrathoracic involvement.

Three patients whose main complaint was severe dyspnoea obtained relief after testosterone treatment. Large effusions were present in each case, bilateral in one (Case 63), and with multiple bilateral parenchymal deposits in another (Case 25). In each case the symptomatic relief was complete, and the patients were able to resume their normal activities. In Case 24 little change in physical signs or X-ray appearance occurred, but in the other 2 cases the effusions were almost completely absorbed, and in Case 25 the pulmonary deposits disappeared (Fig. 1 and 2). Case 24, with widespread recurrences in other organs, relapsed 9 months later, but the other 2 are free of symptoms and signs, and leading normal lives 8 months and 14 months later. Two other patients with dyspnoea as one of several symptoms obtained some relief after treatment. In Cases 3, 10, 24 and 63 the rapidity with which the breathing improved was astonishing, and comparable with the speedy relief of bone pain in the cases mentioned above. In Case 25, however, with dyspnoea at rest, no significant improvement had occurred after a fortnight. Ten oz. of fluid were aspirated from the left pleural cavity with some relief, and during the next two months rapid absorption of the remaining fluid and resolution of the lung deposits took place (Case 25, Appendix). A similar case in which relief of dyspnoea, absorption of effusion and resolution of parenchymal deposits followed removal of both ovaries has been described (Jochweds, Baranowicz and Horecki, 1948), and resolution of parenchymal deposits in a woman of 70 years has been described by Taylor, Slaughter, Smejkal, Fowler and Preston (1948).

Symptoms of intracranial involvement.

Adair (1949) has described a case in which Jacksonian attacks ceased, and physical signs improved, following testosterone administration. In the present series only 2 cases with clear evidence of cerebral metastases have been treated. Case 53, a nullipara, aged 44, remained well for 2 years after radical operation, and then developed frequent headaches and vomiting with progressive ataxia of the left arm and leg. Within a week of testosterone implantation (1000 mg.) the headaches and vomiting ceased, and decided improvement in the power and precision of movement of the left arm and hand took place; the headaches and vomiting did not recur, but 2 months later the onset of severe backache and leg pains marked the beginning of a rapidly fatal downhill course. Case 43, a nullipara, aged 54, had remained well for 9 years after radical surgery when she developed pain over the sternum and double vision. The latter was associated

with a conjugate defect of elevation, thought to indicate a lesion in the anterior part of the oculomotor nucleus. This lesion could have been of vascular origin, or might have been due to a metastasis. That the latter was the more likely is suggested by the development 6 months later of diminished sensation to touch and pinprick over the right trigeminal distribution. This patient took methyltestosterone 50–100 mg. daily for 6 months, but derived non-specific benefit only, the strabismus persisting, and further evidence of intracerebral involvement developing, as already indicated.

Seven cases developed cranial nerve lesions, which most probably resulted from local compression by cranial or dural deposits of growth. Four patients complained of numbness of the lips and tongue, and were found to have diminished sensation to touch and pinprick and deep pressure over areas of maxillary or mandibular nerve distribution (Cases 30, 49, 51 and 54). In addition 2 developed deviation of the tongue, with fibrillation and wasting (49, 51), and at autopsy one of these (49) was found to have hemi-atrophy of the tongue with fibrous replacement. The skull and dura were extensively infiltrated by growth, but a region of compression of the hypoglossal nerve was not found. Unilateral oculomotor palsy, facial anaesthesia and facial palsies were present in 2 cases (Cases 62 and 67), and one case developed unilateral nerve deafness 15 months after commencing testosterone treatment, at a time when widespread evidence of resistance to the hormone was appearing, including multiple bosses on the vault of the skull (Case 37). In none of these cases was any improvement in the cranial nerve affections noted as a result of testosterone treatment.

Objective Evidence of Benefit.

Included in this section are instances of regression, partial or complete, observed in cutaneous recurrences, infiltrated lymph nodes, and localized bony swellings, and of healing of malignant ulcers. The histology of regressing lesions and the X-ray appearances of healing bone lesions are described.

Skin recurrences.

In general skin recurrences show little change during testosterone therapy. This is true of recurrences in mastectomy scars, of lymphatic outspreads, and of distant deposits on trunk, scalp, face or limbs. However, in 2 out of 20 cases (not including those with ulceration) all skin recurrences regressed completely (Cases 20 and 37). In both of these cases, however, the growths were exceptionally sensitive to the hormone, and every other manifestation of disease regressed in as striking a manner as those in the skin (Appendix).

EXPLANATION OF PLATE.

FIG. 1.—*Case* 25. Female, aged 39. X-ray photograph of chest 1 week after subcutaneous implantation of 1000 mg. testosterone, showing left pleural effusion and bilateral parenchymatous opacities due to secondary deposits.

FIG. 2.—*Case* 25. Six months after implantation of 1000 mg. testosterone showing clear lung fields and no effusion.

FIG. 3.—*Case* 44. Aged 48. One week before commencement of methyltestosterone therapy, 50 mg. daily sublingually. Confluent ulcerating recurrence in flaps of mastectomy scar.

FIG. 4.—*Case* 44. Four months later. Healed lesion.

Galton.

Of the 18 other patients with apparently similar deposits in skin, only 2 showed any regression at all, and in both it was partial in extent and of short duration.

Ulcerated lesions.

Eight patients had recurrent skin lesions on the chest wall, with more or less extensive ulceration. The ulceration was usually the result of necrosis in extensive plaques of growth formed by coalescence of multiple enlarging nodules scattered over the chest wall in the skin flaps on either side of the mastectomy scars, but in 3 cases ulceration had occurred in isolated large nodules of growth. In these three cases marked degrees of healing followed testosterone therapy, but in the more extensive lesions healing was complete in only one case (Case 44). The most important single factor delaying healing seemed to be the depth to which the underlying plaque of growth penetrated, and no substantial healing could be expected in those lesions which involved the thickness of the chest wall and had reached the pleura and lung, as had happened in Cases 31, 34 and 56.

The events when a malignant ulcer heals follow a very characteristic sequence, which was observed most completely in Cases 21, 37 and 44. In each case the pre-treatment appearance of the lesions was similar, namely coarsely granular, raised hard edges with more or less purulent slough and crusted exudate in the irregular necrotic granular floor. The first stage of healing is marked by a reduction in the coarseness of the granular nodules of growth, so that the entire lesion takes on a more smooth and uniform appearance. The quantity of exudate and necrotic material becomes less, and the granular appearance becomes finer and more uniform, giving way finally to a smooth glossy surface. At this stage the formerly raised edges are more or less flush with the surrounding skin, and the sharp line of demarcation is replaced by an imperceptible gradation between skin and ulcer floor as the opalescent epithelium grows over the floor from the periphery, following leashes of easily visible fine blood vessels roughly orientated at right angles to the advancing sheet of epithelium. Pieces cut from the edge for histological examination at this stage show much dermal fibrosis, often with hyaline change; in Case 44 no viable tumour cells were found, but clumps of malignant cells, some in mitosis, were present among whorls of collagen in the sections from Case 37, but the lesion subsequently healed completely, and has not again broken down 18 months later although fresh cutaneous and osseous metastases have appeared. In Case 44 the ulcerated lesion occupied most of the chest wall, but healing took place in 6 months when all dressings were discarded (Fig. 3 and 4). Fourteen months later several small nodules have appeared in the upper part of the lesion, and a biopsy specimen from one of these shows carcinoma cells in dense fibrous tissue. The effect of depth of lesion in preventing healing was well seen in Case 64. This was a bun-shaped lesion in the upper end of the scar in a woman of 43. It measured 5 cm. in diameter and 1 cm. high, and was firmly fixed to the underlying chest wall. For 3 months after the commencement of testosterone therapy the lesion showed the early stages in the healing process described above. The centre of the lesion, however, became necrotic and infected, and 1 month later no further healing had occurred. The whole area, together with a portion of the underlying rib and pleura, was excised,

and examination of the operation specimen showed unusually dense masses of fibrous tissue with very few tumour cells. There is no sign of recurrence 6 months later.

Localized bony swellings.

In Cases 4, 17 and 37 localized bony swellings were present before treatment. In Cases 4 and 37 the medial end of the right clavicle was expanded by an exquisitely tender uniform swelling, 4 cm. × 6 cm. in Case 4, and somewhat smaller in Case 37. In Case 17 a hemispherical swelling over the manubrium sterni was present, 2 cm. in diameter. During treatment with testosterone these bony swellings decreased in size, regressing completely in Cases 17 and 37, but coming to a standstill in Case 4, in whom evidence of intrathoracic metastases appeared 5 months later, although the shoulder pains did not recur, and the clavicular swelling was not again tender to percussion. In Case 37 a fresh hemispherical bony swelling appeared 1 year after treatment was commenced over the lateral third of the same clavicle, but the originally present swelling at the medial end has not recurred 20 months later.

Lymph nodes.

Like skin lesions, infiltrated lymph nodes rarely regress with testosterone therapy. In the present series lymph nodes were involved in 30 cases (not counting those treated previously by radiotherapy). Of these, complete disappearance of the enlarged nodes was observed in only one patient, Case 20, who has been mentioned above in connection with regression of skin deposits. In her case the growth seemed particularly sensitive to testosterone, and the complete disappearance in 10 weeks of a large hard node 4 × 4 cm. in diameter and filling the angle between the clavicle and the rat-tailed tendon of sterno-mastoid was very striking. This patient relapsed 11 months later. Case 3, a woman of 37, crippled with multiple bone deposits, who obtained complete relief of pain and regained full mobility of neck and limbs after one month's treatment with methyltestosterone, 100 mg. daily for 8 days, followed by 50 mg. daily thereafter, also showed marked regression of axillary and supraclavicular lymph nodes, though some of these were still just palpable 4 months later.

Case 35 was a Stage IV growth in a woman of 36 years. She had nodes in both axillae. One year previously she had been given radiotherapy to the primary growth and to the axilla, and 6 months previously had been given pelvic irradiation for induction of the menopause. Five months later she was given methyltestosterone sublingually 80 mg. daily for 6 months, during which the primary growth and homolateral and contralateral axillary nodes underwent very considerable regression. The nodes in the opposite axilla disappeared completely, so that the patient had become a " Stage II " case, and was therefore submitted to radical mastectomy. The operation specimen revealed a small scirrhous growth, and one axillary node was infiltrated by carcinoma. Six months after the operation nodes again appeared in the opposite axilla and were treated by irradiation. In 3 other cases temporary softening and some diminution in size of enlarged nodes was observed.

The incidence of regression in the several sites of involvement discussed is indicated in Table VII.

TABLE VII.—*Response to Lesions Involving Different Sites.*

Site of lesions.	Regression.	Partial regression.	Failure.	Total.
Skeletal	8	7	13	28
Intrathoracic	2	3	7	12
Intracranial	0	0	2	2
Skin	4	5	19	28
Chest wall ulceration	1	3	4	8
Lymph nodes	2	3	25	30
Stage IV cases	2	0	13	15

Some Features of Cases with Bone Deposits.

Distribution.

In this series skeletal deposits were mostly multiple, and were associated with pain in 25 out of 31 cases. In 23 of these cases the spine was involved, the dorsal vertebrae being most often affected, the cervicals least often. There were 19 cases with pelvic involvement, and all of these had spinal deposits as well. In 12 cases with extensive pelvic deposits the femora were also involved, bilaterally 10 times, with destruction of most of the shafts in 1 case, in which pathological fracture of the shaft occurred 2 months after implantation of testosterone. Deposits in ribs, clavicles and scapulae have been recorded 14, 5 and 4 times respectively, but may well have been present in other cases. Five cases had erosion of the sternum with tender bony swelling. Precise data are not available with regard to metastases in the skull, but these were known to be present in 5 cases. The results in cases with skeletal involvement are summarized in Table VIII.

TABLE VIII.—*Cases with Skeletal Involvement. Response to Androgen Therapy in Relation to Menopause and to General Clinical Condition before Treatment.*

	I. Good condition.				II. Poor condition.				III. Fair condition.				Total.
	Relation to menopause.			Total.	Relation to menopause.			Total.	Relation to menopause.			Total.	
	Pre-	Artificial menopause.	Post-(natural).		Pre-	Artificial menopause.	Post-(natural).		Pre-	Artificial menopause.	Post-(natural).		
Success	1	1	2	4	2	0	0	2	1	0	0	1	7
Moderate success	2	1	1	4	0	0	3	3	1	0	1	2	9
Failure	..	1	3	4	1	2	5	8	0	0	0	0	12
Radiotherapy as well	2	..	..	2	..	..	1	1	..	..	..	..	3
Total	5	3	6	14	3	2	9	14	2	0	1	3	31

Premenopausal cases with bone deposits.

Of the 31 cases, 3 received palliative radiotherapy to the affected bones (Cases 2, 27, 69) and are not included in the figures. Of the remainder, 8 were premenopausal cases, all but 2 of whom received great benefit from androgen therapy. Three of these were the bedridden patients referred to above, who were able to resume their normal activities within a few weeks of being treated with testosterone, and remained in good health for over 1 year in 2 cases (Cases 3, 7, 37). The fourth was a housewife of 45 with extensive spinal, costal and pelvic deposits, whose activities were drastically curtailed by pain. She also secured complete relief from pain in the month after methyltestosterone therapy was

begun and is well 6 months later (Case 26). Case 4 was a nulliparous woman of 49 complaining of pain in the left hip and right shoulder, the latter exacerbated by the smallest movements of the arm, active or passive. No deposits were seen radiologically to account for the hip pain, but the medial end of the right clavicle was swollen and tender, expanded by a tumour measuring 4 × 6 cm. After taking methyltestosterone 50 mg. daily for one month, the complete range of shoulder movements was restored, the patient was without pain, and the bony swelling no longer tender to percussion, this latter finding being a characteristic result of the treatment. Six months later the swelling is slightly smaller, but intrathoracic metastases have appeared and the general condition has deteriorated. Of the 3 remaining premenopausal cases, 1 (Case 47), a para-2 aged 38, attended hospital 9 months after radical mastectomy with severe anaemia and pains in back, hips and shoulders. She was intolerant to doses of methyltestosterone greater than 30 mg., and although the dose was increased in 5 mg. steps from an initial dose of 10 mg. on 3 occasions the treatment had to be abandoned each time, owing to the sudden onset of headache, nausea, fever, drowsiness and increased intensity of the pains. These symptoms subsided within a day or two on withholding the hormone. Case 66, who presented with paraplegia of 6 weeks' duration, has been referred to above. The last premenopausal case (Case 10), a para-1 aged 45, had had a right radical mastectomy 2 years previously for a lump in the breast present for 1 year. She attended hospital with cough, dyspnoea, pain in the chest and stiff neck. No X-ray evidence of cervical spine involvement was present at the time, but all the symptoms improved after implantation of 500 mg. testosterone. The improvement, however, was short-lived, and 2 months later the pains in the neck returned, and on this occasion radiography revealed extensive destruction of the axis vertebra. Further treatment with methyltestosterone (80 mg. daily) was without value, but the pain quickly responded to local radiotherapy.

Postmenopausal cases with bone deposits.

In contrast to the premenopausal patients in whom testosterone conferred marked benefit in 6 out of 8 cases, only 2 out of 15 patients with skeletal metastases who had passed the menopause responded favourably, although pain was relieved for 2 to 3 months in 5 others. Apart from the postmenopausal state and the slightly greater average age, there was little clinically to distinguish these patients from those who improved so greatly after treatment. The preparations and doses used were similar to those which had been given with success to the premenopausal patients. Both of the postmenopausal patients who were benefited by testosterone were in Group I (good general condition), whereas 2 of the most successfully treated premenopausal patients were in very poor condition before treatment (Cases 3 and 37), and a third (Case 26) was assessed as " fair " (Group III). Four of the 7 failures among the postmenopausal cases were in poor general condition before treatment, and accordingly placed in Group II (Cases 12, 49, 51, 67); three were in good general condition.

The two " successes " were Cases 14 and 52. The former was a woman of 60 who had had a right radical mastectomy $2\frac{1}{2}$ years before. She complained of increasingly severe " rheumatism " in the right hip, and was found to have a large area of rarefaction in the head, neck and intertrochanteric region of the

right femur. She also had 2 large hard fixed parasternal swellings, probably arising in submammary lymph nodes. She was given 100 mg. methyltestosterone daily, and in 3 months' time was walking well and without pain. In addition the parasternal swelling had become greatly reduced in size. Surprisingly she showed on examination 3·5 cm. of shortening of the right femur, but no impairment of movement about the hip-joint, within the limits imposed by the rigidity of her paralysis agitans, and the X-ray revealed an unusual subcapital fracture of the neck with downward and backward displacement of the capital fragment. The second case was of a nullipara aged 51, who had been recurrence-free for 10 years after a radical mastectomy, when she developed backache, which persisted, increased in severity, eventually confining her to bed. Multiple deposits of secondary carcinoma filled most of the dorsal and lumbar vertebrae, and all the pelvic bones. Within a few days of the implantation of 500 mg. testosterone the pain abated, walking was quickly resumed, and she is pain-free 14 months later without further treatment. The nature of the pain is therefore open to question, but the patient gave no history of previous chronic backache, the onset of the pain coincided with the finding of multiple secondary deposits in the appropriate bones, and relief promptly followed implantation of a very small dose of testosterone.

Testosterone was also given to 5 young patients in whom an artificial menopause had been earlier induced (Cases 5, 30, 50, 62 and 70). In the first and last of these cases pain was relieved, but in the first it was never severe, and in the last its recurrence followed the appearance of further metastases.

The difference in response by premenopausal and postmenopausal cases just mentioned was not met with in the series of 70 cases described by Adair, Mellors, Farrow, Woodard, Escher and Urban (1949), or in the larger series of 285 cases analysed by the Council on Pharmacy and Chemistry (1949). In Adair's series 9 out of 48 cases with skeletal metastases benefited from androgen therapy, but no difference in response was noted in premenopausal and postmenopausal cases (12 of the 48 cases were premenopausal, compared with 8 out of 28 in the present series). In the Council's series of 285 cases, the ages ranging from 25–79 years, favourable effects were evidently independent of age. No explanation is offered for this discrepancy.

Post-treatment changes in bone.

It is difficult to account for the rapid relief of skeletal pain brought about by testosterone. Equally striking is the speedy disappearance of tenderness to percussion of affected bones, and the later diminution in size of bony swellings due to subperiosteal deposits. Radiologically all that can be observed are the changes in position and concentration of minerals in the neighbourhood of a deposit following testosterone administration, but these changes are characteristic. They consist in the homogeneous deposition of minerals in the bone surrounding the deposit, so that at first the deposits acquire a more definite outline, and may appear more extensive in size and number. This is a common appearance 2 months after commencing treatment, but soon gives way to a more uniform but rather structureless appearance, as the areas of decalcification in the deposits become filled in from the periphery with deposited minerals. After 6 months this process usually comes to a standstill. It is questionable whether this recalcification confers increased strength on the bones in which it

takes place, for further collapse of vertebral bodies may occur while it is in progress, and in Case 14 early remineralization was occurring at the time of the fracture of the femoral neck referred to above. The illustration of one case in the paper of Hermann, Adair and Woodard (1947) also shows a pathological fracture of the shaft of the humerus in which much sclerosis had taken place. It may in this case have assisted the impaction which " cured " the fracture. Good examples of recalcification in this series are seen in Cases 3, 5, 7, 26 and 37, and in Cases 3 and 60 collapse of more vertebral bodies occurred during the process. Perhaps immobilization during the period of remineralization would prevent such further collapse, weight-bearing being avoided until the process was complete.

Androgen therapy does not of course prevent the development of fresh blood-borne metastases in bone or other tissues. Several authors have noted cases originally free of skeletal deposits, in which these appeared during treatment (Cutler and Schlemenson, 1948 ; Hermann, Adair and Woodard, 1947) ; Adair, Mellors, Farrow, Woodard, Escher and Urban, 1949). The same is true of cases with bone deposits, and in the illustrations in the first two papers just cited fresh osteolytic deposits are present in bones showing extensive recalcification round earlier deposits. Several instances of this phenomenon have been met with in the present series. The paradox is probably to be explained by the fact that the metabolic processes accelerated by the androgen proceed most rapidly at the beginning of treatment, and then slow down to a stationary though raised level. Bony metastases developing some time after treatment has begun are therefore no longer exposed to the same biochemical conditions as those present at the commencement of treatment.

DISCUSSION.

From the therapeutic standpoint the effects of androgen administration resemble but are not superior to those of castration. Dramatic improvement occurs with both methods in a small percentage of cases, and in larger numbers less striking remissions are seen, but most cases do not benefit. In the most favourable cases remissions last for 5 years or more, but in the remainder, recurrence and deterioration in health appear between 6 and 12 months from the start of treatment. With neither method can a favourable result be foreseen ; probably more successes are met with in the premenopausal group, although striking improvements often occur in women who have passed the menopause, sometimes many years previously.

The Results of Surgical Castration for Mammary Carcinoma.

Early work.

Schinzinger (1889) drew attention to the rapid course of the disease in young women as compared with that in elderly women, and suggested that castration in the former might be expected to improve the prognosis by artificially hastening the onset of old age. He does not seem to have put his idea into practice. The young science of bacteriology was advancing rapidly at that time, and it was hoped that infective agents would in time be found as specific causes of most of the major diseases. Several papers appeared at the turn of the century describing intracellular bodies in association with cancer, which were thought to be parasitic

yeasts. In opposition to the parasitic theory of carcinogenesis, Beatson (1896), of Glasgow, published an account of two cases of mammary carcinoma in which striking improvement had followed castration. Beatson believed that carcinoma was the expression of an abnormal kind of lactation in which the mammary epithelium pushed its way outwards into the stroma instead of inwards towards the lumen, there to disintegrate in the form of milk. He had learned of " the custom in certain countries to remove the ovaries of the cow after calving if it is wished to keep up the supply of milk, and that if this is done the cow will go on giving milk indefinitely," and this " pointed to one organ holding the control over the secretion of another and separate organ, and thus explained the absence of that distinct nervous control that I pointed out as characteristic of the mamma." Beatson's first case will be described in some detail, as it illustrates very well the kind of result that may be obtained by testosterone therapy as well as by castration. The patient was a married woman of 33 years, with 2 children, in fair general health, and menstruating regularly. Three years previously she had noticed a lump in the breast while suckling her first child. This increased in size after the birth of her second child 20 months later. When first seen there was a mass $5 \times 3\frac{1}{2}$ inches with ulceration of the skin of the breast over an area of 1 square inch and adjacent skin nodules. Radical mastectomy was performed on 25.i.95, but 3 months later an ulcerated recurrence in the scar was found, and a fortnight later had increased in size to $2\frac{1}{2} \times 3\frac{1}{2}$ inches. The whole chest wall was studded with skin nodules, but no lymph nodes were felt. On 15.vi.95 both ovaries were removed; the left was " cystic," the right normal. By 19.vii.95 the " larger mass was much less vascular, smaller, flatter and altogether less prominent, and the same may be said of all the other secondary foci of disease. The tissues around were also softer and more pliable." By 1.viii.95 the " largest area of disease " measured $1\frac{1}{4} \times 2\frac{3}{4}$ inches. Eight months later regression continued. The patient lived until April, 1899, dying 46 months after castration, with fresh recurrences in skin and spine (Thomson, 1902). The second case was that of a woman aged 40, with massive involvement of the whole breast by carcinoma, with extensive infiltration of skin, axillary and cervical nodes as far as the chin; the opposite cervical nodes were also involved, and she complained of pain in neck and arm. After castration the pain was relieved, and regression and softening of the primary mass and of the nodes occurred, lasting 5 months.

Thus in the first 2 cases in which castration was used, shrinkage of the primary growth, regression of skin deposits and of involved nodes, general improvement, and relief of pain were described. Six years later the method had been used extensively, and its value summed up by Beatson (Morris, 1902) as follows: " It is not easy to say beforehand if the operation will do good; but from the cases recently published by Dr. Abbe, of New York (Thomson, 1902; Abbe, 1901), it would seem that it is not contra-indicated after the menopause . . . the value of an operation may be judged by statistics and by personal impressions. Both may be deceptive, though both may contain in them the germs of truth. I believe that from both standpoints the evidence is in favour of the utility of oöphorectomy in certain cases, in prolonging life and lessening pain. It has never, as far as I know, done any harm, and its mortality has been *nil* . . . the treatment has been placed before the profession, because I felt that

they should judge its effects themselves; and if, in their opinion, after a fair trial, it is found wanting, then I hope it will be abandoned and forgotten." Oöphorectomy passed out of fashion a few years later, and was slowly superseded by "X-ray castration," and in the last 10 years by "medical castration" with androgens also.

Boyd (1900) reported the results of oöphorectomy in 54 cases of mammary cancer. Six cases out of 46 showed remissions lasting for 2 years or more, and 17 out of 46 derived some benefit, but in most of these, recurrences occurred between 6 and 12 months later. With one exception the treatment failed in all the patients who had passed the menopause, and the maximum benefit seemed to be derived by women over 40 and still menstruating. Two years later Thomson (1902) of Edinburgh collected and published the results in 80 inoperable cases castrated by several surgeons. In 40 of the cases some improvement took place, although this was negligible in 11, and only temporary in 11 others (under 12 months' survival following oöphorectomy). In 18, however, decided improvement was recorded, "remarkable" in 4. Thirteen cases survived more than 20 months, and 4 between 40 and 46 months. Of the 29 more favourable cases, 6 had ceased menstruating, and 3 were 70 years of age, and of the 20 women still menstruating, 12 were between 40 and 50 years of age. Thomson concluded that the value of castration, though limited, had been established beyond doubt, but, like Beatson (1896), stressed the unpredictability of the results. He drew attention to the cases in which marked improvement had occurred after the menopause, and although he noted the frequent relief of pain and general improvement following the operation, he considered it to be contra-indicated when metastases were present in the viscera or bones. However, in only 6 of the 80 cases listed is skeletal disease mentioned, and 2 of these were in the group in which the best results were obtained. The limited use and poor quality of the diagnostic radiography of the time invalidates the figures relating to bone metastasis. Thomson also considered that the use of thyroid extract, which Beatson had earlier thought to be an important part of the treatment, was without value.

Early experience of castration at the Cancer Hospital, Brompton, was not so favourable as that from other hospitals. Thus F. B. Jessett "had operated on 5 cases, and admitted that the patients complained of less pain, but in no single case was there any improvement in the local disease, which ran its ordinary course, and the patients died. He had used the X-rays in a few cases . . . but regretted his experience was not that of other speakers, who had recorded such admirable results" (Morris, 1902). Herbert Snow had no better fortune: "some 10 or 12 oöphorectomies had been performed, but never with any useful result; the X-rays had been used for some 20 miscellaneous cases with no better consequence" (Morris, 1902). "The X-rays" mentioned by these surgeons referred to local irradiation of disease, not to pelvic irradiation.

Lett (1905) reported the results of castration in 99 cases of mammary carcinoma in 1905. Of these 23·2 per cent derived marked benefit, and if the patients over 50 years of age were excluded, the percentage of successes rose to 41·3 per cent. The nature of the improvement was similar to that described by previous writers. "In successful cases the benefit has been great, and was mainly shown in relief from pain, marked improvement in health, diminution or even disappearance of the growth, healing of ulcers and prolongation of life." One of Lett's

patients remained alive and well for 5 years after castration, in 15 improvement was maintained for more than 12 months, and 4 others were in good health $4\frac{1}{2}$ years or more afterwards. He also noted that beneficial results occurred sometimes after the menopause.

Bruce Clarke recorded a case in which castration was followed within 6 weeks by rapid subsidence of pain and regression of a chest wall recurrence, the patient being in good health 5 years later. In this case unfortunately the age was not stated, but the original excision of the growth was performed 2 years before castration, and a recurrence excised 1 year before (Lett, 1905).

In spite of the well-documented reports described above, castration for mammary cancer fell out of favour, no doubt because of the absence of precise indications, the large number of poor results and the increasing success obtained by local radiotherapy. Morris (1902) stressed the difficulty of assessing the results, and compared " Dr. Beatson's treatment " with the favourable results claimed for the injection of Coley's fluid (heat killed broth cultures of streptococci obtained from erysipelas lesions, and of *Bacillus prodigiosus*) in cases of spindle-celled sarcoma. He also felt that Beatson's view of the ovarian control over mammary cancer was incompatible with the frequent onset of the disease in elderly women, in whom the ovaries were presumed to be inactive. The difficulty of assessing the value of castration was also pointed out by Michels (1905), and thereafter interest waned, although sporadic reports continued to appear (Torek, 1914).

Recent work.

In recent years attention has again been directed to surgical castration. Herrell (1937) reviewed the records of 1906 women treated for mammary cancer, and of 1011 women in a similar age-group without mammary cancer. He found that in the cancer-bearing group the incidence of complete oöphorectomy before carcinoma was diagnosed was 1·5 per cent, whereas in the non-cancer group it was 15·4 per cent, or ten times as great. These figures implied that castration might have protected a small number of women from the subsequent development of mammary carcinoma.

Arguing on these lines Horsley, late in 1937, performed bilateral oöphorectomy on the first of a series of patients immediately after radical mastectomy, in the hope of deferring the onset of recurrence. By June, 1944, recurrences had occurred in only 4 out of the 25 patients who had been observed for between 9 months and nearly 7 years. Horsley felt that the appearance of metastases had been delayed by castration (Horsley, 1944, 1945). Snapper castrated 22 patients after radical mastectomy, and in addition treated them for 3 months with injections of testosterone propionate 25 mg. thrice weekly, and intermittent courses subsequently. Fifteen of these patients had involved axillary lymph nodes at the time of radical operation. Between 12 and 27 months later 4 had already developed recurrences, all 4 belonging to the group with initial axillary involvement (Stage II cases). Snapper concluded that no significant protection had been conferred by the method (Snapper, 1947).

Ulrich carried out hysterectomy and bilateral oöphorectomy in a woman of 42 with metrorrhagia from uterine fibroids and with bilateral mammary carcinoma with skin infiltration and axillary node involvement. The patient

also received several short courses of testosterone acetate (40 mg. daily for 5 days). The local pain associated with the right breast disappeared after the first course of injections, and some decrease in size of the growths with increased mobility and lessened skin infiltration followed (Ulrich, 1939*a*).

Farrow and Adair (1942) reported a case of mammary cancer in a man of 72, in which marked improvement followed castration. Partial regression of the ulcerated primary growth occurred, there was complete relief from bone pain within 2 weeks, and increased density was described in the osteolytic deposits in ribs, vertebrae and scapula, accompanied by slight elevation of serum alkaline phosphatase. Castration for carcinoma in males was not described by the earlier British workers, though Beatson (1896) felt that the testis exerted the same distant influence on other organs as the ovary. Adair, Treves, Farrow and Schnagel (1945) summarized the results of castration by surgery and by X-rays in 335 females, and in a small number of males. Of 304 women in whom artificial menopause was induced by X-irradiation, 47 (15 per cent) were improved, and 4 out of 31 women surgically castrated were improved. The figures are not quite comparable with those of Beatson (1896), Boyd (1900), Thomson (1902), and Lett (1905), as only premenopausal cases were included, and no patient was over 55 years of age. Of the 51 improved cases in the whole series, 33 were 40 years of age or less. Of the male cases the results of surgical castration were considered to be better in older men, and "as dramatic as in cases of prostatic carcinoma." The patient mentioned above (Farrow and Adair, 1942) remained well for 2½ years. Boger (1946) described the case of a married woman of 27, crippled with pain from skeletal deposits of mammary cancer, in whom relief of pain was obvious within 48 hours of castration. In this case 30 mg. of methyltestosterone was given on the day after oöphorectomy, but in view of subsequent experience with this hormone, it is probable that the immediate effect was due to the oöphorectomy alone.

A case of intrathoracic recurrence in a woman of 40 years, 2 years after radical mastectomy for Stage II carcinoma, in whom relief of dyspnoea was observed 4 days after castration, and during the next 2 months absorption of malignant pleural effusion and resolution of pulmonary parenchymal deposits took place, was reported by Jochweds, Baranowicz and Horecki (1948).

The effects of surgical castration may be summarized by saying that it confers definite benefit on a small percentage of cases of carcinoma of the breast, even in an advanced stage, as shown by relief of severe bone pain, recalcification of areas of destroyed bone, regression of primary and secondary manifestations of disease, and conspicuous improvement in general health lasting usually for several months only, but sometimes for several years. The most favourable cases are those in the premenopausal group in women, though striking improvement occasionally follows castration in older women, and in the small number of cases reported in males the best results are to be expected in elderly patients.

In passing it should be noted that some of the remarkable spontaneous regressions which occur, and which may resemble in all respects those already described, are probably the result of "self-castration," both ovaries being completely destroyed by metastases. One such case, in a young woman with widespread recurrences in bones and skin, in whom rapid deterioration was suddenly arrested and followed by 18 months of sustained improvement, was described by Adair, Treves, Farrow and Schnagel (1945).

The results of X-irradiation of the ovaries in mammary carcinoma.

Irradiation of the ovaries as a substitute for surgical castration was first used by de Courmelles (1909, 1926), whose first report appeared in 1909, the method having been used since 1904. He considered the results superior to those obtained by surgery. Wintz began to practise ovarian irradiation in 1920, and described his results 6 years later (Wintz, 1926). Ahlbom (1930) attempted a statistical analysis of his cases, but as 147 out of 163 cases received treatment to other sites as well as to the pelvis, and his series included very few instances of women in the most favourable age group, he failed to demonstrate any positive effects. Very few clinical data are presented in Ahlbom's paper, and in spite of his obvious anxiety to attain statistical precision, it is perhaps surprising that he could quote no examples of undoubted success similar to those reported so often in cases subjected to surgical castration. Clarkson and Barker (1936) reported a case of mammary carcinoma in a woman of 41 with axillary node involvement, and deposits in a metacarpal, a tarsal phalanx, femur and ilium, in which recalcification of destroyed areas in the skeleton followed ovarian irradiation and general body irradiation. The patient was in good health 5 years later ; the ovarian irradiation was considered to be the main cause of the good result.

Dresser (1936) reported the results of ovarian irradiation in 59 cases with skeletal metastases, having selected this group for special observation because of the dramatic benefit conferred on two women with generalized skeletal and other deposits after they had received small doses (700 r) of X-rays directed to the pelvis for local relief of pain. In both cases the periods ceased, general regression of deposits occurred, with skeletal recalcification, and in the second case multiple pulmonary deposits disappeared ; the patient led an active life for another 3 years, largely symptom-free. Of Dresser's 59 cases, 30 were under 45 years of age, and 29 had passed the menopause. None of the post-menopausal patients derived benefit from irradiation of the ovaries. Temporary pain relief occurred in 13 of the patients under 45, but no regression was observed, but in 9 of these patients the relief of pain was accompanied by marked skeletal recalcification and improvement in general condition lasting from 2–3 years in several instances.

Between 1938 and 1945 Halberstaedter and Hochman carried out ovarian irradiation on 60 patients with mammary carcinoma, most of whom were advanced recurrent cases. Of these patients 34 derived some benefit, not necessarily with objective evidence of regression, lasting from 6 months to 2 years (Halberstaedter and Hochman, 1946).

Reviewing the position Taylor (1938) concluded that there was "a small sub-group of young women . . . in whom artificial menopause has shown striking benefit," and suggested that the ovarian hormones were necessary for the growth of mammary carcinoma.

Selective regression of primary growths and metastases.

Although cases are occasionally seen in which every accessible deposit of growth regresses completely after surgical or X-ray induced castration or after administration of hormones, the commoner occurrence is for some deposits to undergo more marked regression than others. This was noted by the early

observers. Thus Morris (1902) wrote: "cancerous nodules in the same tissue lying side by side are not uniformly affected by Beatson's treatment; lymphatic glands yield less readily than cutaneous or subcutaneous nodules, and Stanley Boyd thinks the supraclavicular glands yield more slowly than the axillary." In the case described by Archer and Cooper (1940), X-ray induced menopause was followed by regression of the primary growth and of the supraclavicular mass, but the axillary mass and pulmonary metastasis remained unchanged. The present tendency is to regard skeletal metastases as the most sensitive to irradiation castration. This is exemplified by Dresser's (1936) series, by the cases reported by Farrow and Adair (1942), Adair, Treves, Farrow and Schnagel (1945), Boger (1946), Hunt (1940), Clarkson and Barker (1936), and Ritvo and Peterson (1944). The last-named authors thought that one-third of selected patients with osseous metastases might be expected to improve, and they do not recommend the treatment for other groups. However, relief of respiratory symptoms and regression of pulmonary deposits have been reported many times (Adair, Treves, Farrow and Schnagel, 1945; Jochweds, Baranowicz and Horecki 1948; Dresser, 1936; Hunt, 1940; Taylor, Slaughter, Smejkal, Fowler and Preston, 1948).

The results of X-ray induced castration may therefore be summarized by saying that in general they resemble those obtained by the surgical method, both with regard to the percentage of successes, the group of cases in which benefit is to be expected, and the nature and duration of the improvement. The discrepancy between the results of the early surgical cases, in which castration was believed to be contra-indicated in the presence of bone metastasis, and the more recent results in which skeletal metastasis is regarded as one of the most favourable indications for ovarian irradiation, is probably adequately explained by the lack of satisfactory X-rays in the early work, so that the extent and incidence of bone metastasis was less accurately known.

The effects of androgens in mammary carcinoma.

Among the first reports of the use of testosterone for mammary carcinoma were those of Ulrich (1939*a*, *b*) and of Loeser (1941). The latter used intramuscular injections and also subcutaneous implants of testosterone propionate. The first 3 cases were recurrence free at the time treatment was begun, but remained so for the next 3 to 4 years, whereas all had been treated by X-rays or surgery for recurrences shortly after the original operation (6 months, 8 months and 2 years respectively). Farrow and Woodard (1942) felt that there was a specially close relation between susceptibility to skeletal metastasis and ovarian activity, and were thus led to use testosterone for cases with bone deposits. They analysed the distribution of skeletal metastases in a group of 1380 cases of mammary carcinoma, and commented on the tendency to develop bone deposits early in the course of the disease in the premenopausal group, and late in the postmenopausal group, so that in the distribution curve there were two peaks of high incidence of skeletal deposits, one in the years preceding the menopause, and a trough of low incidence during the menopausal years.

Their earlier results with testosterone were, however, discouraging. In 3 cases administration of the hormone was rapidly followed by headache, nausea and vomiting, depression, flushing and persistence or even exacerbation of bone pain.

X-rays showed rapid extension of the bone disease. These occurrences were correlated with rapid skeletal decalcification with elevation of serum calcium level, and the authors felt that the hormone should not be used in cases of skeletal metastasis. In subsequent years the same workers reported much better results when larger doses of testosterone propionate were used, and were inclined to attribute their earlier lack of success to inadequate dosage (Adair, 1947). Nevertheless good results have been reported with doses of the same order as were originally employed by Farrow and Woodard, for example, by Fels (1944), and by Schwander and Marvin (1947). Bone metastases were present in the cases of Fels and of Schwander and Marvin, and the most impressive result of the treatment in one of Fels' cases was that the patient, a woman of 34, was able to leave her bed for the first time for 18 months, having received 700 mg. of testosterone propionate in 25 mg. doses given on alternate days.

The case reports published by Adair and Herrmann (1946) illustrate very well the kind of result which may follow the use of testosterone therapy. Weight gains, general improvement, rapid relief of pain previously intractable, regression of primary growths and secondary deposits, and increased calcification of bony deposits were recorded. The following are especially noteworthy :

(1) *The ages of the patients.*—One of the best responses occurred in a woman of 63 years, though the other successes were in women of 47, 44 and 42 years of age. Compare also Taylor, Slaughter, Smejkal, Fowler and Preston (1948), who report and illustrate regression of pulmonary deposits in a woman of 70, and the progress report of the American Council on Pharmacy and Chemistry (Council on Pharmacy and Chemistry, 1949), in which the response to androgens in 285 patients ranging from 25–79 years was evidently independent of age.

In a later paper Adair (1947) reports a dramatic result in a woman of 71 who had been confined to bed for 10 months with severe pain and pathological fractures due to widespread metastases in spine, pelvis and ribs. Three weeks after commencing testosterone therapy she remarked on her improved sense of well being, and 4 weeks later left her bed. Two years and 4 months after she was still up and about and in good health, the only untoward effect being irritation from the enlarged clitoris. This case recalls some of those treated by surgical castration (Thomson, 1902).

(2) *The speed of response.*—In Case 4 relief of pain was evident within 1 week of the first injection, and in Case 3 regression in primary and metastatic growths was noted one month later.

(3) *The favourable effect on skeletal metastases.*—The earlier results of Adair (1947) suggested that the best results of androgen therapy were to be expected in cases with metastases in bone. However, equally dramatic results have been obtained in cases with pulmonary deposits and skin deposits, but no secondaries in bone of the common osteolytic type revealed by X-rays. These results are said to be much less common than in cases with bone deposits. Thus Adair stated: " In only a small percentage of cases is improvement to be anticipated where carcinoma involves soft tissues such as liver, lungs, brain and local skin recurrence. It is true that in a few instances there have been striking improvements where an advanced ulcerating breast cancer with surrounding skin nodules, axillary nodes and neck nodes were involved ; where masses were metastatic to the lungs ; and in one instance where the patient was having convulsions from metastatic disease in the brain . . . most such . . . receive no benefit

aside from the temporary general improvement which frequently comes as a result of testosterone injections. On the other hand, a most striking improvement is obtained in most of those cases having bone metastasis" (Adair, 1947).

In general the earlier results of Adair and his colleagues have been confirmed by others, but it is now clear that the proportion of cases with skeletal metastases which responds is not higher than that of all types of case taken together, although the remissions obtained in these cases are more often of practical therapeutic value, owing to the great disability so commonly associated with osseous metastases. Moreover, the beneficial effects of androgens are not superior to those of surgical castration, and a common mode of action probably underlies both methods, though it is premature to speculate further on this matter.

Cutler and Schlemenson (1948) observed 19 consecutive cases of advanced mammary carcinoma for not less than one year after treatment with testosterone propionate. All the patients died within 14 months after the commencement of treatment, 11 of them received no benefit from the treatment, including 6 with skeletal deposits, but some undoubted improvement took place in 8 cases, transient in 4, but more substantial in the remaining 4. The case with the longest benefit was that of a woman aged 42 years with multiple bone deposits and dyspnoeic from a right pleural effusion. The dyspnoea was relieved a fortnight after testosterone therapy was begun, the effusion was absorbed and the bone deposits became recalcified. The disease again became active 11 months later. In another case, a woman of 58 years, a large hemispherical scalp deposit regressed, and its site became again covered with hair. These authors are careful to point out that in 3 cases skeletal deposits occurred during testosterone therapy, and are anxious to stress the limitations of the method, although fully recognizing its value. They recommend continuing injections until benefit is no longer conferred. In view of the small proportion of cases in which favourable responses are seen, it is not surprising that many workers who have treated small numbers of cases have failed to demonstrate the specific action of androgens in mammary carcinoma. Thus Luft (1948) and Watson and Fetterman (1949) each treated 7 cases without observing any objective improvement.

In an analysis of 285 patients treated at various centres in the United States, and compiled by the Council on Pharmacy and Chemistry, regressions in soft tissue lesions were found to occur in about 20 per cent of cases, and in skeletal lesions in 18 per cent. The response was apparently independent of age, and no support was given to the earlier trends towards increasing the dosage to higher and higher levels. In 20 per cent of the cases which derived benefit, the improvement lasted for 9 months or more (Council on Pharmacy and Chemistry, 1949). Instances in which patients have led useful lives for 3 years or more are by no means uncommon (Adair, 1947, 1949), even when the disease was in a most advanced stage, but in a majority of cases relapse occurs within 1 year of the commencement of androgen therapy.

In practice the chief limitations of androgen therapy are: (1) the poor response in 80 per cent of cases; (2) the impossibility of pre-selecting the 20 per cent of successes; (3) the inevitability of relapse; (4) the expense of the hormones.

At present nothing can be said about the first 3 points. With regard to the last, the expense of treatment can be minimized by using the implantation method in all cases which have been shown to respond favourably to oral or

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injection therapy, by discontinuing treatment in every case in which specific response fails to occur within two months, and by avoiding excessive doses.

All but 5 of the cases here reported were too far advanced to warrant any other form of treatment, but there are many cases in whom androgen therapy and radiotherapy might profitably be given simultaneously, and work on these lines is in progress.

CONCLUSIONS.

Fourteen out of 70 patients with advanced mammary carcinoma (55 recurrent cases, 15 inoperable cases) obtained substantial remissions as a result of androgen therapy.

These remissions permitted resumption of normal activity for more than 6 months in all but one case which is still in remission after 5 months, for more than 1 year in 10 cases, 6 of which are still in good health.

Sixteen other patients obtained some benefit from the treatment, but the regressions were incomplete, and lasted 6 months or less.

Seven out of 31 patients with skeletal metastases obtained complete relief of pain, 4 having been bedridden before treatment. Four are in good health over one year later, 2 between 6 and 12 months, and 1 five months later.

Of the 14 "successes," 5 received implants of testosterone (3 of 500 mg., 2 of 1000 mg.) and 9 received sublingual methyltestosterone (6 of 50 mg. daily, 3 of 50–100 mg. daily). Three patients who originally received implants were later given methyltestosterone, and 2 patients who relapsed were given large doses of intramuscular testosterone propionate, but without benefit. One of them again improved when the injections were discontinued.

Testosterone implantation and sublingual methyltestosterone are considered to be as effective as intramuscular therapy with testosterone propionate. Implantation is recommended in cases responding favourably to a "therapeutic trial" with one of the other methods.

Androgen therapy offers a convenient alternative to surgical castration, but the results are probably no better.

SUMMARY.

The treatment of 70 cases of advanced mammary carcinoma by means of androgens is described, with special reference to the use of sublingual methyltestosterone and of subcutaneous implants of testosterone.

The literature relating to surgical castration, X-irradiation of the ovaries and androgen therapy in inoperable or advanced recurrent mammary carcinoma is briefly reviewed.

Useful remissions are to be expected in 20 per cent of cases when one or other of these methods is used, but the group cannot be pre-selected by clinical means.

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APPENDIX.

Illustrative Case Reports.

The following summaries of 8 case histories illustrate androgen therapy in the advanced untreated case of mammary carcinoma (Cases 3 and 20 " successes," Case 28 a " failure " subsequently responding to oestrogen), in the recurrent case with skeletal metastasis (Case 37 a " success," Case 12 a " failure "), with intrathoracic metastasis (Case 25 a " success," Case 13 a " failure "), and with confluent chest-wall ulceration (Case 44, a " success ").

Case 3.

K. B—, aged 37. One child, 6½ years. Menstruation regular.

June, 1947.—First noticed small mobile nodule in left breast.

June, 1948.—Reported to private doctor. Referred promptly for radical mastectomy.

August, 1948.—Felt stiff down left leg and developed constant pain in small of back.

October, 1948.—Pain so severe that she could not walk without support.

November, 1948.—Pain worse. Fresh pains across shoulders.

January, 1949.—Bedridden.

28.ii.49.—First seen Royal Cancer Hospital; slightest movement of arms or legs distressed her. Kyphotic telescoped spine with lower ribs splayed over iliac crests. Several small hard nodes right axilla, right supra-clavicular fossa. X-rays: Extensive metastases throughout dorsal spine, with compression fractures of dorsal vertebrae 4, 5, 6, 8, 12 and lumbar vertebra 2. Fourth cervical almost completely destroyed, spreading into lamina and base of spine and to body of 5th cervical. Multiple pelvic deposits; upper ends of both femora involved.

Treatment.—Between October and November, 1948, diathermy, radiant heat, ultra-violet light, massage, infra-red treatments without benefit.

28.ii.49.—Methyltestosterone 50 mg. *b.d.* for 8 days commenced.

7.iii.49.—Dose reduced to 50 mg. daily.

Progress.—Pains diminished during first fortnight after taking tab. methyltestosterone, and after 1 month she was pain-free and able to walk short distances without aid. Gained 7 lb. in first 6 weeks. By 27–vi.49 was leading an active normal life, her only complaint being of recurrent crops of pimples on face, chest and shoulders. Amenorrhoeic. She walked normally, showed full range of movements of head, neck and limbs. Kyphosis of spine more exaggerated with gibbus over upper dorsal spine. Soft nodes only in axilla and right supraclavicular fossa. Slight facial hirsuties, but marked seborrhoeic folliculitis with pustules on face, chin, interscapular and intermammary areas. X-rays showed marked increase in density with filling in of many deposits, especially in the pelvis. In the spine further collapse of 11th dorsal vertebra present. Last seen 3.x.49 in excellent health. Therapy discontinued.

Case 20.

M. F—, female, aged 48, 1 child.

First seen 12.iv.48 with complaint of a mass in the left breast believed to date from a blow 2 years previously, and of decline in general health and loss of 1 stone in weight over the past 6 months.

On examination.—Pale, tired-looking woman with signs of recent weight loss.

Local conditions.—Diffuse hard mass occupying whole of left breast disc. Retracted nipple, diffuse skin attachment and deep fixation. Plaque of skin infiltration with several satellite nodules over outer half of the breast. Right breast normal.

Regional nodes.—Large mobile nodes both axillae, and both supraclavicular regions. In the right sternomastoid-clavicular angle was a large partially fixed hard mass 4 × 4 cm. No evidence of distant metastases. No radiological evidence of skeletal deposits.

Treatment.—12.iv.48. Methyltestosterone 8 × 5 mg. daily by mouth commenced.

Progress.—A normal menstrual period began 11 days after beginning methyltestosterone therapy. When seen again on 10.v.48 the mass in the left breast had become softer and somewhat smaller, and the right supraclavicular mass was half its former size and freely mobile. The skin deposits were paler in colour, softer and smaller. In view of this occurrence the dose of methyltestosterone was doubled. On 7.vi.48 she had gained 8 lb. in weight, her periods had not recurred, and the mass in the breast was no longer palpable. None of the nodes was more than 1 cm. across, and the skin deposits had undergone complete regression, being visible only as brownish impalpable mottlings. A section through one of these areas showed only hyaline fibrous tissue in the dermis.

By 12.vii.48 signs of masculinization had appeared, namely slight hirsuties of chin, and lips, and a deepening of the voice. She complained of slight swelling of the feet towards the end of the day. Her weight was 10 st. 12 lb., and she had gained 1 stone in weight since the commencement of treatment. At this time no vestige of primary or secondary growth remained, and the dose of methyltestosterone was reduced to 50 mg. daily, and on 23.ix.48 to 40 mg. daily. On 1.x.48 slight vaginal bleeding occurred, probably representing a menstrual period.

In January, 1949, she complained of slight backache at the end of the day. On examination small soft nodes were felt in both supraclavicular regions, but thought to be of no significance. X-rays of the spine revealed no evidence of metastases, and she was given no additional treatment. However, on 7.ii.49 she attended hospital complaining of severe backache, and on examination the nodes felt previously were found to be hard and much enlarged, numerous deposits not exceeding 3 mm. in diameter were present in the skin of the left breast, and the liver edge, which was thickened, firm and somewhat irregular was felt in the right iliac fossa. One of the skin nodules was

excised, and found to contain loose columns of undifferentiated tumour cells invading dense hyaline fibrous tissue. The original primary mass in the breast was not felt. The dose of methyltestosterone was increased to 100 mg. daily, but deterioration continued.

Case 28.

E. H—, aged 71, nullipara.

Mass present in left breast since 1943 and probably earlier. "Burst" towards end of 1946, and continued to spread and discharge until January, 1948, when patient noticed a small lump on opposite breast, and sought medical advice for the first time.

Condition on 9.ii.48.—Very thin, frail old woman of low intelligence.

Local condition.—Left breast replaced by a hard nodular tumour deeply attached to muscle, and infiltrating skin over it. The upper outer quadrant deeply ulcerated, and the excavated floor heavily infected, the edges red, hard, raised and nodular. The upper edge of the ulcer involved the contiguous axillary mass of nodes which was fixed to the surrounding structures. Nipple retracted and mainly embedded in and replaced by growth. *Right breast*: Atrophic. Hard mass in axillary tail 2 × 2 cm., mobile, lightly attached to skin.

Regional nodes.—Large mass of hard fused fixed nodes left supraclavicular region. Several hard mobile nodes right side and right axilla.

Skin.—Two small nodules 0·5 cm. in diameter in skin of right anterior chest wall.

Treatment.—10.ii.48: Implantation of 5 × 100 mg. pellets fused testosterone.

Progress.—One month later the mass in the right axillary tail had increased slightly in size, as had the mass of left supraclavicular nodes. A hard subcutaneous nodule 1 cm. across, not attached to the skin, was felt over the left scapular region. Five months later it was clear that considerable extension of the primary mass had occurred, and the ulcerated part had encroached further into the axilla.

Treatment with 15 mg. stilboestrol daily by mouth was commenced on 12.vii.48, and by 30.viii.48 marked regression of the primary mass, of the mass in the right axillary tail and of the 3 cutaneous deposits had taken place, though little change in the nodes was noted.

By 26.ix.48 the ulcerated part of the primary growth had completely healed; the mass in the right axillary tail, and the skin deposits, could not be found. She took stilboestrol (15 mg. daily) until 20.vi.49, when an irregular contracted tethered pale scar was all that remained of the left breast. The very hard masses of nodes remained unchanged throughout the observation period of 19 months. By 5.ix.49 several small nodules which had appeared at the periphery of the scar during the previous 3 months were shown to contain active malignant cells.

Case 37.

M. K—, female, aged 37. Nullipara.

1943.—First noticed lump in left breast.

1944.—Left radical mastectomy.

1947.—Onset of pain in right shoulder and neck in January, increasing in severity during the year, and appearing in back, left shoulder and hips towards the end of the year.

Nov., 1947.—Confined to bed owing to severity of pain, and its exacerbation by slight movements of head, trunk or limbs.

16.xii.47.—Admitted to Royal Cancer Hospital.

On examination she was in good general condition, but bedridden with crippling pain. There were 3 ulcerated recurrences along the left mastectomy scar, the two larger measuring 2 × 4 and 5 × 3 cm. respectively, with irregular thickened raised edges, fixed bases and coarsely nodular floors. The opposite breast was uniformly hard, fixed to pectoral fascia, and to the skin by multiple points of attachment. There

was extensive involvement of right axillary and both supraclavicular groups of nodes, and a tender diffuse bony swelling of the medial end of the right clavicle. There were 3 skin metastases, one 3 cm. in diameter in the scalp over the left parietal region, two smaller deposits, one in the left lumbar region, one in the left groin. Radiologically there was extensive infiltration of the entire vertebral column, and there were multiple deposits in the ribs, clavicles and pelvis. Mobility of the head and neck and of the right shoulder-joint was limited to a few degrees in all directions by pain, and the slightest active movement of the legs aggravated the neck pain. The left shoulder was affected to a lesser extent.

Other investigations.—Hb 86 per cent, W.B.C. 12,800, serum calcium 12·0 mg./100 c.c. Serum alkaline phosphatase 49·2 units, serum phosphate 3·3 mg./100 c.c.

Treatment.—For the first few days in hospital bed rest, analgesics and sedatives alone were given. The pains were usually relieved by Mist. A.P.C. and nepenthe (g. 30).

31.xii.48.—500 mg. testosterone was implanted in the subcutaneous abdominal fat in 10 pellets of 50 mg. fused testosterone.

Progress.—Two days after the testosterone implantation the pains were distinctly better, the nepenthe mixture was no longer necessary, and during the subsequent fortnight she became well enough to get up and walk. From then on she continued to improve, and at the end of 3 months had regained full movements of head and neck, spine and shoulders. By the middle of April she had gained 9 lb. in weight and was leading a normal life. Menstruation, which had ceased after the implantation, recurred on 19.iv.48, and on 29.iv.48 a further 1000 mg. testosterone in 10 pellets of 100 mg. was implanted.

During this period the ulcerated chest wall recurrences were undergoing slow regression; the skin deposits on trunk and scalp regressed completely, the latter leaving only a flat hairless plaque of skin. The right breast had become normal in consistence and mobility, and the attachments to the skin over it could no longer be demonstrated. The clavicles and vertebral spines were no longer tender to moderate percussion; the supraclavicular nodes on both sides diminished greatly in size, but the right axillary nodes remained unchanged, though no longer tender. X-rays showed increased calcification round many deposits.

By August, 1948, two of the ulcers on the chest wall had healed, but the third, although flatter, had increased slightly in diameter, and shortly after the patient complained of pains on the left side of the neck, shoulders, back and hips. These pains were found to be relieved by 50 mg. of oral methyltestosterone daily, but again recurred when the dose was reduced to 30 mg. daily.

However, by 5.iv.49 there was marked deterioration in general and local condition. She had lost 12 lb. in 9 months, had recurrence of low back pain; the mass in the upper inner quadrant of the right breast was larger, as were the axillary nodes; the bony parasternal swelling in the left 4th interspace had ulcerated through the skin. There were numerous hemispherical bony projections on the vault of the skull, on the frontal bone, on the right clavicle. X-rays showed many fresh osteolytic deposits in spine, ribs and pelvis, side by side with old sclerosed deposits, which were less dense than before.

Injections of testosterone propionate 100 mg. 3 times weekly were begun, but one month later there was no improvement and hormone therapy was discontinued. Her condition rapidly improved thereafter; she was given a short course of palliative X-rays to the lumbar spine and to the fungating chest wall deposit. When last seen on 6.ix.49 she was pain-free, well and active, and the right breast mass and axillary nodes were much smaller, and the bony projections on skull and clavicle were no longer felt.

Side effects of testosterone.—In this patient the side effects of the testosterone therapy were less marked than in many others, although she was under the influence of androgens for 16 months. Facial hirsuties was minimal, and there was no voice change, and no

oedema of the ankles. However, general increase in skin thickness occurred, especially over the face, neck and back, its texture became more coarse, and it was more greasy than before. In the fortnight following the second implantation she complained of pruritus vulvae, and pustular acne appeared on the face, though the more common diffuse inflammation of the sebaceous duct orifices was not marked. There was marked hypertrophy of the clitoris, and an increase in the amount of perineal hair. The redistribution of subcutaneous fat reduced the feminine contours of the body to some extent, and this, together with the change in skin texture, probably accounted for the changed facial appearance noticed by her friends.

Case 12.

V. C—, nullipara, aged 52. Menopause at 45.

1945.—Radical mastectomy for Paget's disease of nipple.

June, 1948.—Onset of backache. Metastases in dorsal spine. Pain temporarily relieved by radiotherapy.

July, 1948.—Recurrence of pain, now involving neck, hips and both thighs.

X-rays.—Extensive destruction of pelvic bones and femora by metastases.

23.ix.48.—Implantation 1400 mg. (14 × 100 mg.) testosterone.

Progress.—No relief of pain.

Case 25 (Fig. 1 and 2).

M. G—, aged 38, nullipara.

11.viii.44.—Left radical mastectomy for Stage I carcinoma of breast. Post-operative radiotherapy.

Remained well until April, 1948, when first noticed breathlessness on effort, which subsequently became worse.

June, 1948.—Signs of large left pleural effusion. No other clinical evidence of recurrence. X-ray chest: Large left-sided effusion. Multiple small opacities both lung fields. Pleural fluid: opalescent straw-coloured fluid. Total protein content 4·45 mg./100 ml. Histology of deposit: Many malignant cells, some of them grouped to form tubules; secondary adenocarcinoma.

Treatment.—29.vi.48: Implantation of 10 pellets of 100 mg. testosterone.

Progress.—For the first month after implantation dyspnoea was still increasing, and the patient was unable to continue with her work as telephone exchange supervisor, but during the second month rapid improvement ensued, and she was back at full work 6 weeks after implantation. She has remained symptom-free since, the signs of effusion became gradually less, and on 3.ii.49 the only abnormality found was slightly diminished expansion at the left base. Radiologically there is now only obliteration of the left costophrenic sulcus, and the parenchymal opacities are no longer visible. She was last seen on 25.viii.49.

Side effects.—Cessation of menstruation from 29.vi.48 to 17.i.49. Periods since regular. Seborrhoeic folliculitis noted 26.viii.48, huskiness of voice 23.ix.48, loss of hair from both temples 18.xi.48.

Case 13.

L. C—, nullipara, aged 39. Menstruation regular 5/21, onset 15 years.

27.ix.48.—Left radical mastectomy for painless lump present 1 month. Single hard axillary node.

10.iii.49.—Several small subcutaneous nodules in mesial skin flap of scar found.

21.iii.49.—Rapidly increasing dyspnoea during past fortnight. Loss of appetite. Large left pleural effusion. Fluid contained malignant cells.

Treatment.—21.iii.49. Radiotherapy to skin recurrences commenced.

30.iii.49.—Methyltestosterone 50 mg. daily commenced.

28.iv.49.—Dyspnoea not relieved. Aspirated from left pleural cavity 32 oz. fluid. Dose of methyltestosterone increased to 100 mg. daily.

5.v.49.—Further 3 pints fluid aspirated from pleural cavity.

Progress.—Steady deterioration. Methyltestosterone was discontinued on 21.vi.49. By 12.ix.49 widespread deposits in skin, lungs, skeleton and liver.

Case 44 (Fig. 3 and 4).

E. M—, aged 48.

June, 1943.—Subradical mastectomy at 12th week of pregnancy for lump in left breast first noticed several months earlier.

1946.—Noticed several small nodules in neighbourhood of scar, soon followed by others, gradually increasing in size with confluence and spreading ulceration.

10.vii.48.—Admitted to Royal Cancer Hospital complaining of recent loss of weight, and swelling of left arm.

On examination.—Good general condition. Local condition: Multiple areas of ulceration with confluence over a wide area on either side of the mastectomy scar extending to the apex of the axilla above and to the level of the 8th rib in the mid-clavicular line below. Most of the raw surface, which was finely granular, was covered by thick crusts of solid exudate, and the edges of the ulcer were only slightly raised above the level of the surrounding skin, and had not the appearance of a rapidly growing neoplasm. Several shotty cervical nodes on both sides. Enlarged right axillary nodes.

Treatment.—The crusts were soaked off with warm saline, exposing the entire ulcerated surface. Soft paraffin gauze dressing.

16.vii.48.—Tablets methyltestosterone 50 mg. daily commenced.

Progress.—Menstrual periods ceased after commencement of treatment. Within 5 weeks the surface of the edge of the ulcerated areas had merged with the adjacent skin surface, and in places opalescent epithelium could be seen growing over the surface of the ulcer, now uniformly smooth. By 23.ix.48 large areas had become epithelialized from the periphery, and by 28.x.48 only one small area about 1 cm. square in the upper part of the ulcer was unhealed. The left arm was less swollen, and the patient had gained 21 lb. in weight. There was marked seborrhoeic folliculitis on the skin of the back, and some larger pustules on the chin. There was coarsening of the facial skin, but no marked hirsuties and no voice change. The dose was reduced to 35 mg. daily. By 2.xii.48 the entire area was paler in colour, the young skin over it thicker, but there was still one small discharging area. This dried up during the next two months, dressings were discarded and therapy discontinued, but on 1.ix.49 two hard pinkish nodules a few millimetres in diameter appeared at its margins, sections of one of which showed active scattered carcinoma cells in fibrous tissue.

Addendum.

Since the manuscript was completed M.K. (Case 37) has attended (28.2.50) with recurrence of pain, M.G. (Case 25) is still in remission (16.2.50). The lesions of E.M. (Case 44) was unchanged on 8.12.49, and K.B. (Case 3) was in remission on 13.2.50, having had a second course of methyltestosterone 50 mg. daily since 12.12.49 for recurrence of right shoulder pain and stiffness. V.C. (Case 12), L.C. (Case 13) and M.F. (Case 20) have died.

THE RELATION OF HISTOLOGY TO SPREAD IN INTESTINAL CANCER.

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IT is well known that different histological types of cancer vary in their rate of growth and liability to metastasise, but this relationship between histology and spread is easier to study in some organs of the body than in others. Cancer of the intestine provides exceptionally good material for investigating this question because surgical operations are planned to remove a good stretch of bowel above and below the tumour as well as a wide field of lymphatic drainage in the mesentery. It happens, therefore, that the pathologist is presented not only with the tumour, but also with its surroundings, so he can first classify the tumour according to its histology, then measure the extent of local spread by direct continuity, and finally record the number and position of lymphatic metastases.

When the histology and spread are compared in this way it soon becomes obvious that different histological varieties often behave differently, but a fairly big series of cases must be examined in order to express these differences quantitatively and give them anything like precise significance. It takes a long time to collect many cases of cancer of one organ of the body such as the bowel at a general hospital admitting patients of all sorts, but this is done much more quickly in a special hospital like St. Mark's, which is intended primarily for diseases of the rectum and colon. In the last 21 years (1928–1948 inclusive) 3,220 operation specimens of intestinal cancer were examined and dissected in the laboratory of St. Mark's Hospital, the general distribution being as follows: 2,642 cases of cancer of the rectum, 429 of the colon, 51 of the anal canal, and 98 cases of multiple malignancy. In each case the records describe the extent of local spread and the number and position of all lymphatic metastases. In preparation for this paper I have re-examined the sections of all these tumours and compared their histology and their local and lymphatic spread. Since rectal cancer is much the largest of these groups, forming 82 per cent of all cases, it will be convenient to start with this.

Histological Varieties of Rectal Cancer.

The three main histological varieties of rectal cancer are adenocarcinoma, colloid (or mucinous) carcinoma and an undifferentiated variety which I shall call "anaplastic carcinoma simplex."

Adenocarcinoma is much the commonest variety, constituting 85 per cent of all cases. Its histological characteristics are well known, and it is sufficient to point out that though the growth usually shows a tubular or acinar pattern there is as a rule very little evidence of mucous secretion, either in the cells or

glandular spaces. Since adenocarcinoma is such a large group it is useful to subdivide it according to the degree of differentiation of the tumour cells into three groups : (1) low grade of malignancy, (2) average grade, and (3) high grade of malignancy.

Colloid or mucus secreting carcinoma is the second commonest variety of rectal cancer, constituting 13 per cent of cases. It has a similar basic structure to adenocarcinoma, but differs in that a large quantity of mucus is secreted. When the mucus is stored in individual cells these have a signet ring appearance but, if the secretion is extra-cellular, tumour cells may be relatively few in numbers, being seen only floating in lakes of " colloid " or scattered round the margin. The secretion is not actually " colloid," of course, but the tumour has long been known to surgeons as a colloid carcinoma, though no doubt mucoid or mucinous carcinoma is a more accurate description.

Carcinoma simplex.—This term is used to describe an anaplastic type of carcinoma composed of polygonal or spheroidal cells destitute of any glandular arrangement, and not showing mucus secretion. The tumour cells are scattered about singly or in small clusters, or they may be arranged in a solid trabecular or alveolar pattern. It may be difficult to decide whether such a growth is a sarcoma or carcinoma, but some part of the tumour generally has features characteristic of carcinoma. These tumours are of a high grade of malignancy, metastasize early and have the worst prognosis of all. A distinctive feature of carcinoma simplex is a disposition to spread by continuous permeation of submucous lymphatics well beyond the visible boundaries of the growth. Fortunately this group is the smallest, constituting only 2 per cent of all cases of rectal cancer.

Relation of histology to spread in rectal cancer.

Cases of rectal cancer treated by radical excision have been divided into four groups according to the extent of spread of the disease as revealed by the examination of the operation specimen. In the first or initial stage the growth is limited to the bowel, the patient at this stage being described as an "A" case. The second stage is reached when the malignant tumour has spread by direct continuity into adjoining structures, but not yet given rise to lymphatic metastases. At this stage the patient is described as a " B " case. The third stage is reached when the malignant growth has spread still further and given rise to lymphatic metastases. Patients at this stage are known as " C " cases. If the malignant growth has spread to distant organs, such as the liver, this is described as the fourth stage.

All the cases of rectal cancer in this series have been classified in this way and the records make it easy to compare the extent of local and lymphatic spread in different histological varieties of cancer. Thus Stage 1 or A cases were present to the extent of 15 per cent in adenocarcinoma (337 amongst 2252), whereas they constituted only 8 per cent in colloid carcinoma (27 amongst 357). Moreover, lymphatic metastases were found in 50 per cent of the cases of adenocarcinoma, 70 per cent of colloid, and 82 per cent of anaplastic carcinoma simplex. The average number of lymphatic metastases in adenocarcinoma was only 4, but in colloid carcinoma 6 and in carcinoma simplex 8. This may be taken as evidence that colloid carcinoma of the rectum spreads more rapidly than adenocarcinoma, and that carcinoma simplex spreads even more rapidly than colloid carcinoma.

Colon Cancer.

The same histological varieties of cancer are met with in the colon as in the rectum and in closely similar proportions. Of the 429 cases in this series, 84 per cent were adenocarcinoma, 14 per cent colloid, 1 per cent simplex and 1 per cent carcinoids. The last two varieties were too few in number for analysis, but comparisons between adenocarcinoma and colloid showed once more that mucus secreting or colloid tumours spread more rapidly than adenocarcinoma. Thus, at the time of operation, only 5 per cent of the colloid carcinomas were still Stage 1, i.e. limited to the bowel, whereas 20 per cent of adenocarcinoma were still found to be at this early stage of development. On the other hand, lymphatic metastases were found in 44 per cent of colloid carcinoma, but in only 36 per cent of adenocarcinomas. One may conclude from this that both in the rectum and colon the local spread of colloid carcinoma is more rapid, and that colloid growths metastasize earlier than adenocarcinoma and therefore have a worse prognosis.

Before turning to cancer of the anal canal, which has an entirely different histology, it is worth pointing out that the conclusion reached with regard to the bad prognosis of colloid carcinoma is rather paradoxical and unexpected. It might have been supposed that a tumour which secretes mucus would grow more slowly than one which appears to have lost this function and devoted itself exclusively to reproduction. This may be because the mucus or colloid material tends to ooze along and distend tissue spaces and lymphatic channels thereby facilitating the spread of neoplastic cells. In other words, the colloid acts as a lubricant. It is certainly true that one sometimes finds small quantities of colloid material in lymphatic glands which do not contain neoplastic cells, suggesting that this has made its way along in advance.

Anal Cancer.

There were only 51 examples of anal cancer in this series, approximately 1·5 per cent of all operation cases, but this figure does not represent the true incidence or distribution because at one period in this 20-year survey many cases of anal cancer were treated by radiation. These 51 cases have been divided into three groups: (1) transitional cell carcinoma arising from the upper part of the anal canal, 49 per cent.; (2) squamous cell carcinoma derived from the lower part of the anal canal, 37 per cent; and (3) mixed tumours showing both squamous or transitional cells and adenocarcinoma, 14 per cent.

I have compared the extent of local and lymphatic spread in these three groups, but the differences between them were slight and of doubtful significance. Metastases were found in the ano-rectal glands in 48 per cent of transitional cell carcinoma, 37 per cent of squamous and 40 per cent of mixed tumours, but some of the squamous cell tumours derived from the lower half of the anal canal may have given rise to undiscovered metastases in the inguinal glands. So it would seem that these different histological varieties of anal cancer do not differ to any important extent in their local and lymphatic spread.

CONCLUSION.

In conclusion it should be pointed out that the relationship between histology and spread of tumours is easier to study in some organs of the body than in others. There are many reasons for this. In the first place it is obvious that

such relationship between histology and spread can only be established if the histological varieties are clearly distinct: as definitely different, let us say, as adenocarcinoma, colloid carcinoma and carcinoma simplex of the intestine. This is seldom the case. Another handicap arises from the fact that in some organs of the body the injury caused by a malignant growth is much more attributable to its anatomical position than to any niceties of histology. This is particularly true of tumours of the kidney, ureter and bladder. Then again in some organs of the body the path of local and lymphatic spread of carcinoma is variable or difficult to follow. So it is not to be expected that conclusions drawn from the study of intestinal cancer can be extended as easily to other organs. None the less it is probably true that each histological variety of cancer has its characteristic tempo of spread, even though the pathologist is seldom able to express it precisely.

A COMPARATIVE STUDY OF RADIOTHERAPY TECHNIQUES.

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FAILURE in the treatment of malignant disease is most often due to the occurrence of metastasis before treatment of the primary lesion is instituted. There are, however, a number of cases in which the tumour remains localized for a considerable time, but cannot be removed surgically on account of its size and anatomical situation, and cannot be destroyed by radiotherapy without irreparable damage to neighbouring normal tissues. It is the aim of the radiotherapist to exploit the differences in sensitivity between normal and neoplastic tissues, and it is the object of this research to see whether the effects of radiotherapy on certain tumours can be enhanced by modification of the techniques of therapy in common use at present.

A course of X-ray treatment involves a number of variable factors. Among these are the number and size of separate doses into which the treatment is divided (fractionation); the time spacing of individual fractions; the total dose given; the time over which it is spread (protraction); the intensity of irradiation during each dose, and the quality of the radiation used, depending on the voltage and filtration.

Clearly, it would be impossible to obtain intelligible results from varying more than one of these factors at a time, and in this investigation we have studied the effect of varying the fractionation.

This work is reported here, not so much for the results obtained, as to illustrate a method of investigation which may prove to be of value.

METHOD.

Tumours, even of similar histological type, vary so much in their response to irradiation that it is of little value comparing the effects of different techniques of treatment on different tumours, even though these tumours appear to be very similar histologically.

It was therefore decided that comparisons of technique must be made on one and the same patient in each instance. For this purpose accessible tumours were selected that could be accurately divided into segments by appropriate skin markings. The segments, usually 2 or 4 in number, and as nearly as possible of equal size, were then treated by one or more experimental techniques, while one segment was treated by a control technique. Segments not being treated were protected by lead shields while neighbouring segments were irradiated, and care was taken to avoid overlap of adjacent segments or an untreated gap

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between them by careful skin markings and "setting up." The question of side-scattering of X-rays from one segment into adjacent ones was investigated by physical measurements with micro-ionization chambers in a wax "phantom." The amount of scattered radiation was found to be less than 5 per cent at 1 cm. from the edge of a treated segment, and this was considered small enough to be ignored.

In this way erroneous conclusions resulting from unpredictable individual variations in response are largely eliminated, and each case constitutes a complete separate experiment. The results of individual experiments can later be compared to see whether the experimental technique in question regularly produces a particular effect.

The tumours and surrounding skin were examined at frequent intervals to follow progress, and to detect and note differences in response between the areas. In addition, biopsies were taken from all areas before, during and after treatment. At first, weekly biopsies were taken, but it soon became obvious that in the earlier stages more frequent examinations were necessary, while after completion of the course of treatment intervals of a month or more might elapse. The sections were examined by a pathologist who not only had no preconceived views about the various techniques of irradiation employed, but was kept in ignorance of the area from which each specimen had been taken.

Biopsies from each area before treatment were studied carefully to provide a base line, and also to give an idea of the degree of variation between different areas of the same tumour. This variability was often found to be marked, and differences in biopsies subsequently taken from the several segments had often to be discounted as representing no more than possible chance variations. The changes usually described were seen in the biopsies as treatment proceeded, and the possibility of cell counts on the lines suggested by Glucksmann (1947) was considered to see if numerical comparisons of the efficacy of treatment could be made. However, the variation naturally occurring in different areas of the same growth, among other things, led us to abandon attempts at assigning figures to each biopsy as too difficult and time-consuming to carry out conscientiously and as liable to erroneous interpretation. Instead, a general impression of the changes, both in the tumour cells and in the stroma, was gained from the examination of each section, and only when favourable changes were regularly found to be more advanced in biopsies from any one area was the technique there employed regarded as superior on histological grounds.

Experimental Techniques Investigated.

Conventional techniques of X-ray therapy of malignant tumours vary in detail in different centres, and according to the type of tumour treated. Fractionation is, however, usually by equal daily doses given on 5 or 6 days each week, and this fractionation technique is applied with little discrimination to widely different sorts of tumour.

Certain theoretical considerations, mentioned below, suggested that a different method of fractionation, leaving longer intervals between the fractions, might be more effective. Two experimental techniques were therefore chosen for trial; in each the protraction was at the rate of about 1,000*r* tumour dose per week, but the size of individual fractions and the intervals between them were varied. Technique I consisted of 500*r* given twice a week; Technique II consisted

of 1000*r* given at weekly intervals; whilst 200*r* were given 5 days a week as the control technique.

It will be seen that both the experimental techniques chosen involve longer intervals between the applications than is usual. An important direct effect of a dose of irradiation is temporary suppression of mitotic activity. Koller (1948) found that in squamous- and basal-cell carcinomas of the skin the number of dividing cells was considerably reduced 6 hours after 200*r*, and that after doses of 400*r* and 500*r* mitotic suppression might last for several days. The susceptibility of cells to irradiation damage varies at different periods of their life cycle, being greatest in early prophase (Lea, 1947), and irradiation given during the period of mitotic suppression is relatively ineffective. Spear and Glucksmann (1938, 1939) have demonstrated by experiments on irradiation of tissue cultures and animal tissues *in vivo* that a greatly enhanced lethal effect is obtained if fractions are spaced so as to coincide with the recovery of mitotic activity following the suppressive effect of the previous dose.

Information concerning the duration of mitotic suppression in human tumours after different doses is scanty. It probably varies in different tumours, but will depend largely on the size of the dose. A clinical investigation indicating advantages from a wider spacing of fractions has been reported by Koller and Smithers (1946).

Koller (1948) considers that the nature of the tumour bed and the indirect effects of irradiation on the stromal reaction in the surrounding normal tissue exercises an important influence on the response of the tumour itself. An important feature of this stromal reaction is an infiltration by plasma cells and lymphocytes. These cells are highly radio-sensitive, and too-frequent doses of irradiation may well interfere with their activities.

Clinical Material.

The necessity for accurate division of the tumour into segments and for serial biopsies limited our choice to accessible tumours. A further limitation was, of course, imposed by consideration for the patient's welfare, and no case was undertaken where it was felt that there would be less chance of effecting a cure by the method.

Tumours of three types were treated—large rodent ulcers (2 cases), carcinomas of the skin (5 cases), and carcinomas of the breast involving the skin (4 cases).

Most tumours of the first two types are radio-sensitive, and a successful result could normally be expected from the use of an orthodox technique. These tumours were treated in order to assess the relative efficiency of the experimental techniques, and to determine any differences in the reaction of normal tissue at the edge of the tumour.

The cases of carcinoma of the breast were of special interest, for, as is well known, a considerable proportion are markedly radio-resistant. The cases selected had either local plaques of recurrent growth in the region of a previous mastectomy scar or large fungating tumours unsuitable for surgery where only a palliative result could be hoped for.

Case Reports.

Three cases will be described in detail, to illustrate the application of the method, and the remainder summarized more briefly.

Case 1.—J. H—, male, aged 38. Squamous-cell carcinoma.

History.—For ten years a warty growth had been present on the lower part of the left buttock, near the anus. In April, 1947, it began to grow rapidly, bled easily, and an offensive discharge developed. A biopsy taken four months later showed invasion by squamous-cell carcinoma.

Surgical excision was attempted, but bleeding was excessive despite the use of the diathermy needle, and as the tumour appeared to extend deeply the operation was abandoned and the patient was referred for radiotherapy.

On examination.—There was a warty cauliflower-like tumour on the lower part of the left buttock, extending forward onto the left side of the perineum, and measuring 10 × 6 cm. The surface was ulcerated and infected, and the base

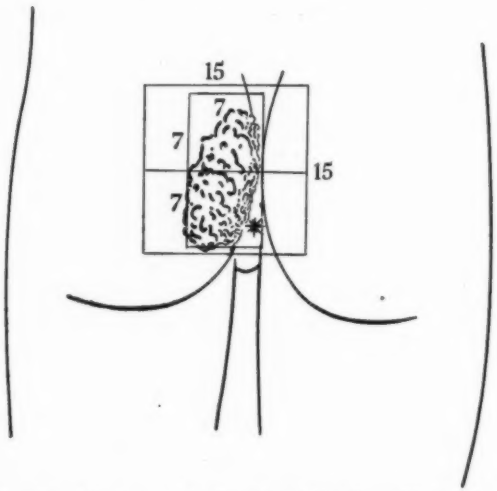


FIG. 1.—The site of the tumour in Case 1 and the disposition of the upper and lower treatment fields.

was broad and somewhat indurated. The anus and rectum were clear of the growth, but some extension up into the left ischio-rectal fossa was palpable. The regional inguinal glands were palpable, but were not thought to be invaded by growth.

Treatment plan.—The tumour was divided into upper and lower halves, which were treated by two adjacent fields of $7\frac{1}{2}$ × 15 cm. each, arranged as in the diagram (Fig. 1). Later, as the tumour shrank, these were replaced by two adjacent 7 × 7 cm. fields, the same line of division between the two halves of the tumour being observed.

Area 1 : (the upper half) was used as the control, and was treated by 200*r* daily, 6 days per week.

Area 2 : (the lower half) was treated by experimental Technique II—1000*r* at 7-day intervals.

Physical data.—200 K.V. X-rays ; filter 1 mm. Cu + 1 mm. Al. ; H.V.L. 1·3 mm. Cu ; F.S.D. 50 cm. ; dose rate 40*r* per minute.

Treatment of both areas was started on September 5, 1947. By September 27, both areas had received 4000*r*, and treatment was suspended. A good deal of tumour still remained, however, and treatment was resumed on October 6th, a further 1000*r* being given to each area.

Summary of Clinical progress.—During the first three weeks of treatment the tumour shrank in size, and the smaller fields were substituted on the 11th day. The surface infection cleared up, projecting cauliflower processes flattened out, and a clean pink granulating surface resulted. The usual erythematous reaction developed, and proceeded to moist desquamation over the tumour and surrounding skin. The area of skin at the periphery which was only covered by the original $7\frac{1}{2} \times 15$ cm. fields showed pigmentation, but no desquamation. The dose to this skin area had only been about 2000*r* when the smaller fields were substituted. No difference in reaction or tumour response in the two areas was apparent at this stage.

On October 6, as viable tumour was still feared to be present, further treatment was given as described.

Three weeks after the conclusion of treatment a marked difference was apparent between the two areas. The area treated by the control technique (Area 1) was completely healed over by healthy new epithelium except for a small ulcer at the site of the first surgical attack. The remaining tumour consisted of some firm pink papillary processes, almost flush with the surface.

In Area 2 much of the tumour and surrounding skin covered by the treatment applicator was still raw and denuded of epithelium. The remaining tumour was a little flatter than in the control area.

Eight weeks after treatment further flattening was apparent. Area 1 was healed except for the remains of the ulcer. Area 2 showed very little progress of healing, although a few islands of epithelium had appeared in the raw skin area ; there was some discharge from a fissure between two sessile processes.

Four months after treatment a striking difference between the two areas was apparent. The control area was healed and showed marked pigmentation, all that remained of the tumour being some fibrotic nodularity beneath the surface. Area 2 showed up as a sharply outlined, completely unpigmented square, corresponding to the treatment applicator. There was still an indurated fissure at the site of the tumour, and an area of skin below the tumour remained unhealed and had clearly undergone necrosis.

Five and a half months after treatment the patient complained of burning pain in the lower part (Area 2), and an increase in discharge from the fissure which was still present. A month later (April, 1948) radionecrosis was obviously developing in Area 2 ; the fissure had deepened, its edges were grey and necrotic, and the surrounding tissues were pale and avascular, and as hard as cartilage. By May a large necrotic ulcer corresponding to Area 2 had developed. Local treatment failed to bring about healing, so in November, 1948, the whole treated area was excised, after a preliminary colostomy. This was done partly because residual foci of active growth were suspected. By the beginning of 1949 the wound was healing rapidly, with no sign of recurrence locally or in glands. By September, 1949, healing was complete except for an area 1 cm. in diameter. The colostomy was not yet closed.

Summary of histological findings.—The pre-radiation biopsy showed invasion by well-differentiated keratinizing squamous cell carcinoma with very little

mitotic activity. There was a moderate degree of associated inflammatory reaction. Biopsies taken from both areas during and after treatment showed progressive differentiation of the tumour cells with reduction in the percentage of viable cells and their final disappearance. By the end of active treatment only granules and clumps of keratin lying in dense fibrous tissue indicated the remains of the tumour. No sign of tumour could be seen in the biopsies taken at $6\frac{1}{2}$ months, and no significant differences between the series of sections from Areas 1 and 2 were detected. The material from the surgical excision in November, 1948, was carefully examined; many sections were prepared, but no trace of residual or recurrent carcinoma was found. Dense fibrosis and considerable areas of radionecrosis were seen, together with marked endarteritic changes in the larger blood vessels deep in the specimen.

Comments.—The control technique and Technique II appeared equally effective in destroying the tumour cells. The important point illustrated by this case is the induction of radionecrosis by Technique II (1000*r* weekly). This was already apparent in the skin in the treated area within a few weeks of completing the treatment, although necrosis of the tumour bed did not develop until about 5 months later.

Whether Technique II would have been successful with a smaller dose without inducing necrosis is open to question; even with a smaller total dose this technique is considered to be dangerous.

Treatment was followed by keratinization of most of the tumour cells and a bulky growth remained for a considerable time, giving a clinical impression of residual, viable tumour, though no viable cells were recognizable histologically.

Case 2.—A. P—, female, aged 56. Recurrent carcinoma of breast.

History.—In December, 1935, a radical mastectomy had been performed for carcinoma of the right breast. The original growth, a spheroidal cell carcinoma, had been adherent to the skin and pectoral muscle, but the axillary glands were not involved. A small nodule of recurrent growth (1·25 cm. diameter) had appeared, adjacent to the mastectomy scar, in February, 1945. Superficial X-ray treatment was given (4 daily doses of 800*r*) and the nodule regressed, being quite healed by September, 1945. Two years later a further recurrence developed at the same spot.

On examination.—There was a firm plaque of recurrent growth just lateral to the upper part of the right mastectomy scar, and adherent to the skin, but there was no actual fungation and no axillary or other glands were involved clinically. An area of slight scarring with some telangiectasis marked the site of the previous X-ray treatment.

Treatment plan.—The plaque was divided into two areas, upper and lower halves, each being treated by a 4 × 4 cm. field.

Area 1: (the upper half) was treated by the control technique—200*r* daily, to a total of 3000*r*.

Area 2: (the lower half) was treated by Technique I—500*r* twice a week, at 3- and 4-day intervals, to a total of 3000*r*.

Physical data.—95 K.V. X-rays; filter 1 mm. Al.; H.V.L. 1·65 mm. Al.; F.S.D. 25 cm.; dose rate 113*r* per minute.

Summary of clinical progress.—Treatment was started on November 17, 1947. Within ten days some regression of the tumour plaque was apparent, and appeared

to occur more rapidly in Area 2. At the end of treatment almost all the tumour had regressed, leaving a very small central ulcer. There was a uniform erythematous reaction, and no difference was now detectable between the two areas.

Three months after treatment some induration was palpable in Area 1, but was thought to be due to scarring from the previous biopsies. Two months later, however, a definite nodule of recurrent growth was apparent in Area 1; Area 2 still remained healed and with no sign of recurrence.

In June, 1948, further X-ray treatment was given to the recurrence in Area 1, but this time Technique I was employed, in view of its success in Area 2. Four weeks later the nodule had practically disappeared, leaving a small ulcerated area. Nine weeks after this further treatment a small radionecrotic ulcer had formed; this healed slowly, but was still present in August, 1949, at which time the ulcer margins were quite soft and there was no sign of recurrence.

Summary of histological findings.—The degenerative irradiation changes in this spheroidal cell carcinoma with a densely fibrous stroma were less easy to follow than in other more cellular tumours, but in the biopsies taken six weeks from the beginning of treatment it was apparent that these changes were more advanced in Area 2. No tumour cells were recognizable in the biopsy taken from Area 2 eleven weeks after beginning treatment, whereas definite resting tumour cells were seen in sections from Area 1 taken at the same time. Five and a half months after the beginning of treatment the section from Area 1 showed an area of recurrent growth (Fig. 2). Further sections of this area during the subsequent course of radiotherapy (Technique I) showed degeneration and disappearance of the tumour cells.

Comments.—The previous X-ray treatment in 1945 made it necessary to give a lower total dose than would otherwise have been chosen in this case.

Technique I appeared to be superior to the control in that response occurred earlier, the reaction was no more severe, and the growth has not recurred in two years, whereas recurrence began to develop in the control area after three months. Treatment of this recurrence in Area 1 by Technique I appears to have been successful, though the repeated treatment has caused some necrosis.

Case 3.—E. H—, female, aged 69. Carcinoma of breast.

History.—A lump in the upper part of the left breast, first noticed in 1945, had grown considerably and was beginning to invade the skin by the autumn of 1947. In view of the extent of the growth she was referred for palliative radiotherapy.

On examination.—Before treatment there was a large plaque-like tumour present in the upper outer quadrant of the left breast, measuring 11·5 × 9·5 cm. The skin was invaded over practically the whole extent of the tumour, and fungation was imminent. The tumour was fairly mobile, there being only slight attachment to the pectoral muscle. There was no palpable involvement of the regional lymph glands, apart from one small gland in the pectoral chain close to the tumour.

Treatment plan.—The tumour was divided into four areas, as shown in the diagram (Fig. 3).

Area 1: Technique I (500*r* twice a week), total dose 3680*r*.

Area 2: Control (150*r* daily), total dose 3000*r*.

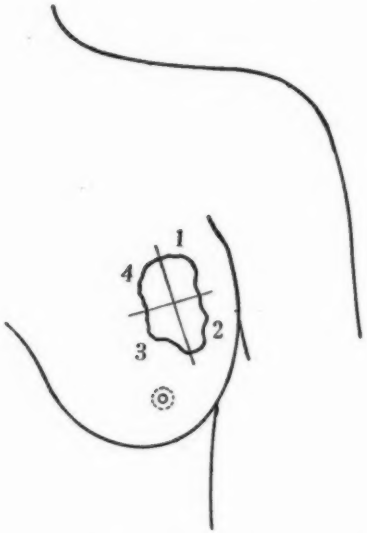


FIG. 3.—Carcinoma of breast (Case 3) divided into four areas.

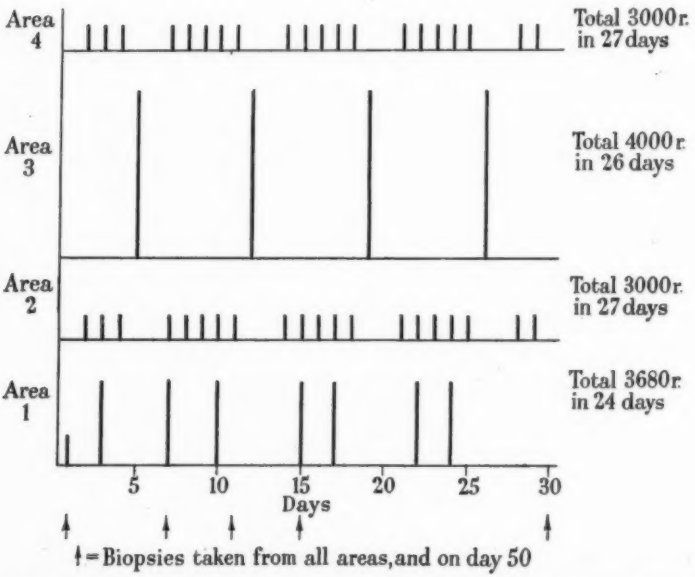


FIG. 4.—Case 3. Chart indicating the treatment of the four areas; the course had to be curtailed on account of local reaction. The height of the vertical lines is proportional to the dose given at each treatment.

Area 3 : Technique II (1000*r* once a week), total dose 4000*r*.

Area 4 : Control (150*r* daily), total dose 3000*r*.

Physical data.—200 K.V. X-rays ; filter 1 mm. Cu + 1 mm. Al. ; H.V.L. 1·3 mm. Cu ; F.S.D. 50 cm. ; dose rate 40*r* per minute.

The discrepancies in total dose were occasioned by severe local reaction and infection which necessitated curtailing the treatment to the control areas and to Area 1. The control technique was modified in this case, 150*r* being given daily instead of the usual 200*r*. This was done to bring it more into line with the average daily tumour dose administered in this clinic for carcinoma of the breast. The dose to Area 1 by Technique I was 3500*r*, the extra 180*r* having been delivered before the treatment plan was finally settled. These points are illustrated on the Treatment Chart (Fig. 4). These differences in total dose and periods of protraction, unfortunately, somewhat detract from the significance that can be read into the findings in this case.

Summary of clinical progress.—Treatment was started on January 20, 1948. During treatment the tumour shrank slightly, but there was no difference observable between the four areas. By the end of treatment, February 18th, the greater part of the surface was covered by smooth granulation tissue. There was still a considerable amount of tumour present, with a subcutaneous shelf of induration palpable all round the edge. There was severe erythema of the surrounding skin, with much pain and discharge, and for these reasons treatment was curtailed. It is probable that the unusual severity of the reaction was due to infection of the raw surface, as the patient had fever up to 101° F. with rigors. These symptoms settled rapidly with penicillin and sulphonamides given systemically.

One week after treatment there was moist desquamation over the whole treated area. A fortnight later the skin had healed all over except in Area 3, where there was still a sharply outlined unhealed area corresponding to the treatment applicator. The reaction in Area 1 was more marked than in the control areas, but it had received a larger dose. The tumour had shrunk to 10·5 × 6·5 cm. The ulcer floor was covered by necrotic slough and its edges were still firm, but were softer and less raised in Areas 1 and 3.

Seven weeks after treatment the skin had begun to heal in Area 3. The skin in Area 1 showed well-marked pigmentation, but was perfectly healed. Well-marked differences were now apparent in the different areas of the tumour, which had shrunk further to 9 × 6 cm. Both areas treated by the control technique showed clinical evidence of active residual tumour, the ulcer in these areas having a hard raised edge with much adjacent subcutaneous induration. Area 1 showed some suspicious induration, but less than in the areas treated by the control technique. Area 3 was soft and nearly flat, with no definite sign of residual growth. The centre of the ulcer was occupied by a slough which was removed under anaesthesia at the same time as the biopsies were taken from all four areas.

Three months after treatment there appeared to be little progress in healing, and as active growth seemed to be present in Areas 2 and 4, a radical mastectomy was carried out. This was now fairly easily performed owing to the shrinkage of the tumour, and the patient has remained well since with no sign of local recurrence or metastasis up to September, 1949.

Summary of histological findings.—The pre-radiation biopsy showed a

spheroidal cell carcinoma with a moderate amount of fibrous stroma. During treatment the percentage of degenerating cells increased in all areas, but more so in Areas 1 and 3 than in those treated by the control technique. Four weeks after treatment sections from Areas 1 and 3 showed a markedly fibrous stroma in which there were occasional scattered cells, thought to be viable ; Areas 2 and 4 on the other hand, both showed a considerable amount of active tumour still present. There was much the same degree of inflammatory reaction in all areas.

The mastectomy specimen showed marked naked eye and microscopical differences between the areas. In both the areas treated by the control technique there was a thick white fibrous mass extending to a depth of 3 or 4 cm. from the surface, whereas in the experimental areas the thickness of the tumour residue was much less, only about 1 cm. Microscopically, sections from Area 1 showed much young fibrous tissue with occasional degenerate tumour cells, but no sign of viable growth. In Areas 2 and 4 there was a thick mass of fibrous tissue in which there were numerous tumour cells, many degenerate, but a considerable number almost certainly viable. Area 3 showed a layer of fibrous tissue with scattered degenerate tumour cells, together with a few that were thought to be viable. As in Case 1, several occluded blood vessels were seen deep in the operation specimen (Fig. 5). It was noted that the surviving tumour cells tended to be at the back, or deep aspect of the tumour, a finding which limits the deductions that can be drawn from superficial biopsies.

The remaining 8 cases consisted of the following :

Case 4.—F. H—, female, aged 58. Rodent ulcer.

This patient had a large basal cell carcinoma involving the back of the ear and adjoining scalp. This was divided into four areas and treated with 200 K.V. X-rays as follows :

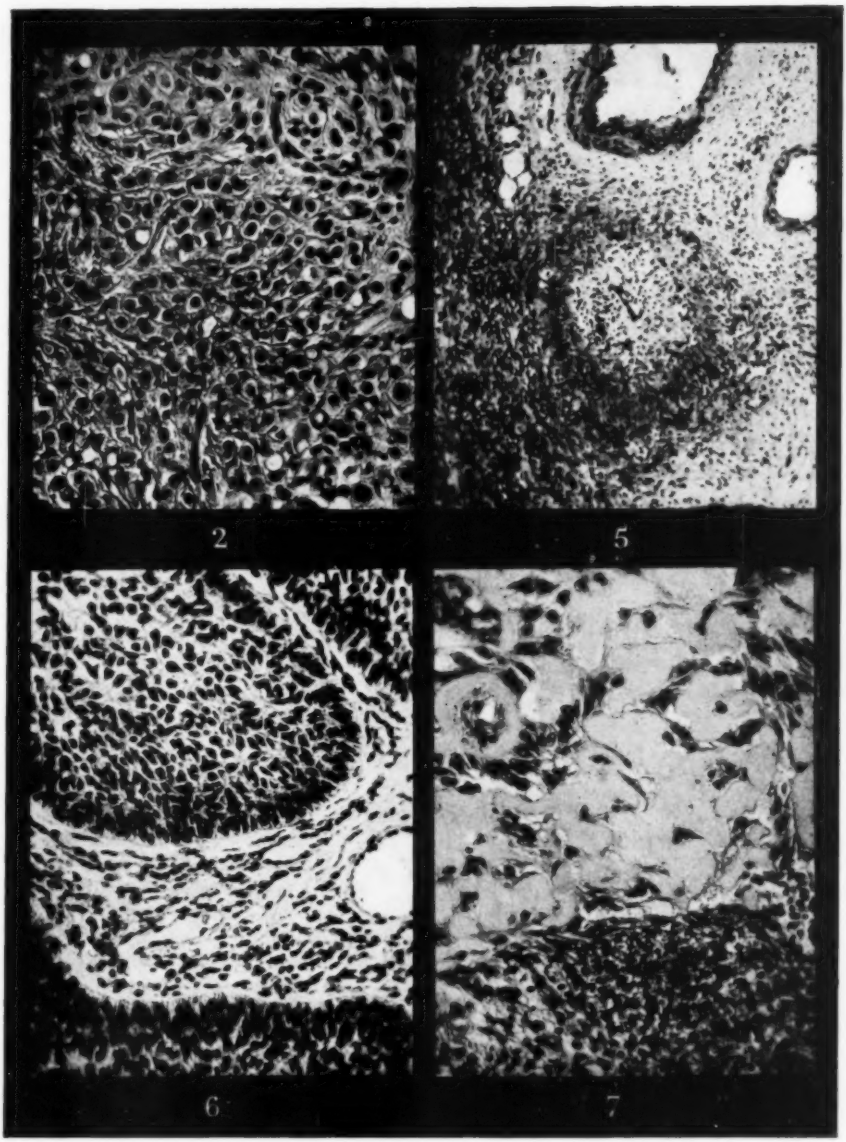
Area 1 : Control (200*r* daily), total dose 4000*r* in 24 days.
Area 2 : Technique I (500*r* twice a week), total dose 4000*r* in 26 days.
Area 3 : Technique I to a total dose of 5000*r* in 33 days.
Area 4 : Technique II (1000*r* once a week), total dose 4000*r* in 22 days.

It will be noticed that Area 3 had a higher total dose and longer treatment time ; this was for the purposes of a future investigation and the results have not been taken into account in the present series.

Biopsies taken during treatment showed an apparently satisfactory response in all areas, with no obvious advantage in any technique. The high total dose and close proximity of the four fields undoubtedly resulted in overdosage. Six

EXPLANATION OF PLATE.

FIG. 2.—A nodule of recurrent growth from Area 1 in Case 2. × 220.
FIG. 5.—An obliterated blood vessel from the deep part of the operation specimen (Case 3). × 55.
FIG. 6.—Biopsy of the rodent ulcer from Case 5 before treatment ; a solid mass of tumour cells occupies the upper half of the field. × 220.
FIG. 7.—A similar area to that shown in Fig. 6 at the end of treatment. The tumour cell mass in the upper part of the field shows extensive hyalinization, and there is heavy lymphocytic infiltration of the stroma. Note that the magnification is the same as in Fig. 6. × 220.
FIG. 8, 9 and 10.—Squamous-cell carcinoma of the ear (Case 8). Biopsies taken before, at about the middle of, and at the end of the course of treatment. No tumour cells remained in the final biopsy (Fig. 10), only keratin, surrounded by foreign-body giant cells. × 220.



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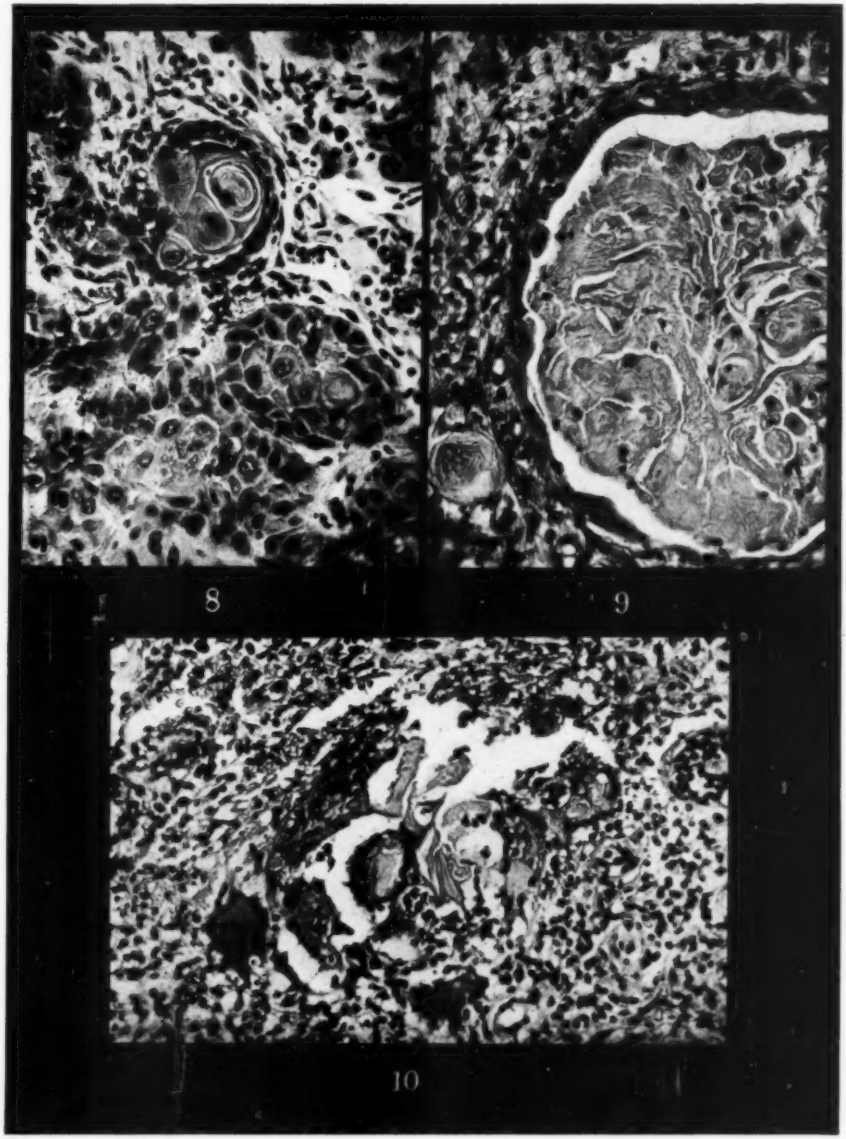

Sambrook, Thackray and Woodyatt.

weeks after treatment radionecrosis developed in the centre, and extended to involve part of the cartilage of the ear. This healed slowly, but 18 months after treatment recurrent tumour developed, involving Areas 3 and 4. This had to be dealt with by radical excision. This late recurrence was quite unexpected; at the original treatment the whole lesion had been covered, including a generous margin of healthy skin, and dosage had been higher than that normally needed to cure rodent ulcer. It seems possible that this is an example of the supralethal effect described by Paterson (1948).

Case 5.—J. B—, male, aged 67. Rodent ulcer.

This man had a very large basal cell carcinoma of the skin of his back, which was treated by 95 K.V. X-rays in two halves as follows:

Area 1: Technique I (500*r* twice a week), total dose 4000*r* in 29 days.

Area 2: Control (200*r* daily), total dose 4000*r* in 28 days.

The biopsies in this case showed progressive hyalinization of the tumour cell masses (Fig. 6 and 7). Clinically there seemed to be only a very slow response to irradiation, with little shrinkage of the growth, and in the absence of histological control one would have concluded that the treatment had failed to produce much effect. The response to Technique I was less satisfactory than to the control technique, and even after fifteen months there was still a suspicion of viable growth in Area 1, although the tumour as a whole had shown a progressive regression. Recently, twelve months after treatment, there have been recurrences in both areas.

Case 6.—M. F—, female, aged 67. Carcinoma of breast.

A very advanced tumour, fungating at several points, and with hard enlarged glands in both axillae and supraclavicular triangles. Treatment was purely palliative. The breast tumour was treated by 200 K.V. X-rays in upper and lower halves by two pairs of opposed glancing fields. The upper half was treated by Technique I to a total of 3500*r* in 24 days, and the lower half was treated by the control technique, 200*r* daily, to a total of 3500*r* in 23 days.

Clinically the tumour shrank more rapidly in the control area. There was some difference in the skin reactions in the two areas; simple pigmentation in the lower half, but marked dry desquamation in the upper area.

The biopsies before treatment showed a spheroidal cell carcinoma. During treatment well-marked irradiation changes developed in both areas; there was no significant difference between the response to the two methods of treatment.

Case 7.—B. D—, female, aged 68. Recurrent carcinoma of breast.

A local recurrence in the mastectomy scar 18 years after the removal of the breast for carcinoma. The upper half was treated by Technique I (500*r* twice weekly) to a total of 3500*r* in 24 days, and the lower half by the control technique (200*r* daily) to a total of 3600*r* in 24 days; 95 K.V. X-rays were used. Pre-radiation biopsies showed a carcinoma composed of comparatively regular uniform cells with areas of well-marked tubule formation; subsequent biopsies showed an earlier favourable response in the upper half. Clinically the response was equally satisfactory in both areas.

Case 8.—A. P—, male, aged 76. Carcinoma of ear.

This patient had a large exophytic squamous-cell carcinoma of the ear. The upper half was treated by the control technique (200*r* daily) to a total of 4000*r* in 28 days. The lower half by Technique II (1000*r* once a week) to a total of 4000r in 22 days; 200 K.V. X-rays were used. Clinically the response was equally satisfactory in both areas, and there has been no recurrence in the treated area since (2 years), although an enlarged gland in the neck has had subsequent treatment.

Histologically there was an earlier favourable response in the control area. No viable tumour cells could be found in either area in the final biopsies, only keratin masses, often surrounded by foreign-body giant cells (Fig. 10). The preliminary biopsy, and one taken about the middle of treatment showing marked differentiation, are shown in Fig. 8 and 9.

Case 9.—J. T—, male, aged 85. Carcinoma of cheek.

This patient had a squamous-cell carcinoma of the skin of his cheek. Upper half—technique I (500*r* twice a week)—to a total of 4500*r* in 32 days. Lower half—control technique (200*r* daily)—to a total of 4600*r* in 35 days; 200 K.V. X-rays were used; clinically and histologically both areas showed an early favourable response, and there was no apparent difference between the two areas. There has been no recurrence since treatment.

Case 10.—D. B—, female, aged 58. Carcinoma of skin.

A squamous-cell carcinoma on the dorsum of the wrist. Upper half—Technique I (500*r* twice a week)—to a total of 3500*r* in 22 days. Lower half—control technique (200*r* daily)—to a total of 3600*r* in 25 days; 200 K.V. X-rays were used. Clinically there was rapid regression of the lesion in both areas, and the tumour had disintegrated completely before the course was finished. The serial biopsies showed a very satisfactory response in both areas, no viable cells remaining within two weeks of starting treatment. The number of mitoses decreased as treatment proceeded, and the mitotic figures soon became grossly abnormal, but none of the biopsies appeared to have struck a period of complete suppression. There was little to choose between the two areas, but the response in the upper half was considered to be slightly more favourable. It was noteworthy that the fibroblastic stromal reaction and the inflammatory cell infiltration were equal in the two areas, being in no way adversely influenced by the larger individual doses in the upper half.

Case 11.—C. G—, male, aged 74. Carcinoma of ear.

This patient had a squamous-cell carcinoma on the helix of his ear. Upper half—Technique I (500*r* twice a week)—to a total of 4000*r* in 25 days. Lower half—control technique (200*r* daily)—to a total of 4000*r* in 27 days; 95 K.V. X-rays were used. Tumour regression occurred rapidly in both areas, and the biopsies showed marked squamous differentiation in response to irradiation. There was no difference—clinically or histologically—between the two areas.

DISCUSSION.

The evaluation of the various techniques used in these experiments is no easy matter. Treatment of a malignant tumour by an ideal technique of radiotherapy should ensure destruction of all the tumour cells, while leaving the adjoining normal tissues relatively unharmed; and this in the shortest time and with the fewest possible applications. These points all require consideration; destruction of tumour cells, speed of action, and ease of treatment being weighed against the occurrence of unfavourable reactions, local or general, immediate or delayed. Clear-cut results are obtained when the tumour recurs in one area and not in others, and ideally the experiment should be continued as long as necessary to allow for this possibility. It may be more difficult to reach a conclusion if there is no recurrence, or if recurrences are seen in both areas, though differences detected clinically and histologically may still give some indication as to which is the more favourable technique.

It is obvious that in this investigation we have only touched on the fringe of the problem. At the outset we mentioned a series of factors involved in a course of radiotherapy, and so far we have varied only one of these, and that only by two large variations in the same direction. Bearing in mind that the ideal technique may well be different for each type of tumour, it is obvious that much remains to be done. Any conclusions we may draw from this small series of cases must be purely tentative; the final evaluation of these techniques may require the elapse of a considerable interval of time, and in view of the possibility of variation in different parts of the same growth many cases must be studied before definite conclusions can be reached. Complete equality of total dose and protraction were seldom obtained, and this introduces another variation from strict experimental conditions. With these facts in mind the following conclusions are cautiously presented.

Technique I (500*r* twice a week) was used in 9 cases; 3 squamous-cell carcinomas, 4 carcinomas of the breast, and 2 rodent ulcers. The clinical result in the 3 cases of epithelioma was uniformly good, and in one case this technique gave slightly quicker results than the control. In 2 cases of carcinoma of the breast this technique gave a better response, judged clinically and histologically, than the control; in the other 2 cases the results were equally good. The cases of basal cell carcinoma responded better both clinically and histologically to the control technique (daily fractions of 200*r*).

Technique II (1000*r* once a week) was tried in 4 cases; 2 squamous-cell carcinomas of the skin, one carcinoma of the breast, and one rodent ulcer. With the possible exception of Case 8 (carcinoma of skin) it produced more severe skin reaction, and in 2 cases this went on to radionecrosis. This damaging effect on normal tissues, compared with the control technique, condemned it as unsuitable. In addition, it did not appear any more effective than the control in the cases of epithelioma, and in the cases of carcinoma of the breast and rodent ulcer it failed to kill all the tumour cells.

This investigation is being continued and it is intended to try different fractionation techniques from those described here. Even if the experimental techniques were to prove only equal in efficacy to the orthodox control, a course of treatment involving X-ray applications only once or twice a week would have considerable practical advantages over the more usual daily applications.

SUMMARY.

A method for comparing the effects of different techniques of radiotherapy on one and the same tumour is described.

The results of treating a small series of cases by various methods of fractionation are presented and tentative conclusions are drawn.

We wish to acknowledge the help and encouragement of Professor B. W. Windeyer, who provided facilities for the clinical part of this work to be carried out in the Meyerstein Institute of Radiotherapy, Middlesex Hospital. Part of the expense of this investigation was defrayed by the British Empire Cancer Campaign.

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THE ROLE OF CONNECTIVE TISSUE IN THE RADIATION REACTION OF TUMOURS.

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IT has been asserted that an improvement of results in the treatment of cancer can only occur when the disease is diagnosed and treated in its early stages, or when new sources of radiant energy, more powerful and penetrating than have hitherto been used, are applicable. Such an assertion is in itself a confession of defeat. It presupposes that with the present-day methods of radiotherapy the maximum results obtainable have been achieved, and implies that the results in early stages are satisfactory, and the burden of improvement in the treatment of cancer is put on the early diagnosis. It is suggested in the present paper that a better understanding of the biological behaviour of tumours undergoing radiation, particularly that of the relationship between tumour and tumour-bed, is the basis on which further improvement in treatment can be expected. Clinical and experimental evidence is at hand to show that the destruction of tumours is caused, not by the killing of all malignant cells as a direct effect of radiation, but that the various changes which are induced by radiation in the whole tumour bearing non-malignant tissues play a major role in this process.

The importance of tumour-bed and stroma reactions is shown, for instance, by the fact that in some cases of carcinoma cervix uteri, 3 to 6 months after completion of radium or X-ray treatment, apparently active tumour cells can still be seen, yet those cases have been found to be five or more years' cures. Quantitative cytological analysis of radiation effects in hysterectomy specimens of cancer of the cervix uteri, in which dose and effect could be related, has shown that in one particular case tumour cells were fatally injured, and that tumour parenchyma was completely broken up by a radiation dose of 1700 r, which is far below what is usually considered a " tumour lethal " dose. Recurrences of carcinoma of the cervix are sometimes found to be situated in regions which received the maximum radiation dose (10,000–20,000 gamma röntgens). Evidence is at hand which clearly indicates that the difference in radiosensitivity between a primary tumour and its lymph-node metastases is mainly due to the difference in the histological organization of tumour-bed and stroma at the primary and secondary sites. The fact that the topography of tumours exercises an influence on the outcome of treatment will not be denied by anybody with clinical experience. It is well known to radiotherapists that *ceteris paribus*, neoplastic tissues respond differently to radiation in the absence or presence of oedema and infection in or around the tumour. The radiosensitivity is directly proportional to the degree of anaplasia of a tumour, yet the eradication of such tumours can only rarely be achieved by present-day methods.

These few instances are selected from many clinical and pathological findings to illustrate that the environment of tumours is of great importance, and must be taken into consideration when devising improvements in radiotherapeutic procedures. It may prove to be important not only in surgical and radiotherapeutic practice, but also as regards theoretical problems of oncology. The classical study of Willis (1948) emphasizes the fact that potentially neoplastic tissue zone is much greater than the size of the initially appearing tumour would indicate, and brings forward evidence which lends support to the field-theory of the origin of cancer. Thus the possibility that the " recurrence " of a tumour after surgical treatment may represent an entirely new cancerous change in a predisposed tissue, and is not due to the incomplete removal of the malignant growth, has to be considered. The aim of post-operative X-ray therapy is not only to destroy viable tumour cells which might have been left *in situ*, but also to reduce the chance of the occurrence of an entirely new malignant growth by producing gross alterations in the histological architecture of particular regions, showing a proneness to neoplasia, and to affect the substrate in which any malignant cell left *in situ* might progress and defeat the treatment. The importance of connective tissue and tumour-environment has also been stressed by Vernoni (1948), whose views on the connective-tissue role in carcinogenesis find support in recent research work.

A better understanding of the behaviour of the connective tissue of the tumour-bed and stroma during and after radiation treatment leads to the revision of fundamental principles on which the rationale of treatment is based. The practical application of the latter in the radiotherapy of accessible tumours finds expression in a new technique, described as the " sieve or alternating chess-board method " (Jolles, 1949*a*). For the past two years this method has been tested on a small number of patients, yet the radiation response in these cases has shown characteristics which hitherto have not been seen in tumours irradiated by the usual conventional methods. The purpose of the present paper is to describe these phenomena, and to discuss their bearings on the destruction of malignant growth.

The Biological Basis of Radiation Effects in Tumours.

The radiation-induced changes in the cellular and stromal part of the tumour may be summarized briefly as follows: The cellular injuries consist of diverse nuclear or chromosome injuries, as well as disturbances in cytoplasmic enzyme activity. The chromosome injuries manifest themselves during mitosis which follows treatment, and lead to cell death by the breakdown of the mitotic mechanism. The amount of chromosome injury and the number of injured cells show a direct dependence on the dose. Radiation-induced enzyme disturbances, the extent of which also depends on the dose, either lead to reversible injuries, e.g. suppression of mitosis, delay in spindle formation, stickiness of chromosomes, etc., or result in a gross impairment of cytoplasmic organization which becomes manifest as an abnormal increase in the size of the cell (monster-size cell). It is also claimed that radiation induces cell-differentiation. " Differentiation " is defined as an internally or environmentally conditioned adaptation of the cell for a specific function. While such an event can occur in some tumours spontaneously, there is no proof that ionizing radiation induces cell-differentiation in malignant tissue. The analysis of very diverse types of

tumours has shown that the increase in the number of differentiated cells is only relative, being caused by the destruction of undifferentiated cells, as a result of which the differentiated cell nests become more distinct. It should also be emphasized that a " hyper-keratinization " of tumour cords after radiation is an abnormal phenomenon, and there is no justification whatsoever to describe it as " radiation-induced differentiation." We define such a change as a particular type of cell degeneration. The recent investigations by Bloom and Jacobson (1948), Anderson (1949), and Warren and Dixon (1949), brought forward further evidence to show that radiation does not produce cell differentiation.

In the stroma and tumour-bed two chief events can be distinguished : hypertrophy of the connective-tissue elements, and a reaction closely resembling inflammation. The first phenomenon mainly consists in an excessive deposition of intercellular collagen fibres (fibrosis) ; the second is represented by an invasion of lymphocytes, polymorphonuclear leucocytes and macrophages into the fine network of connective tissue forming the stroma and tumour-bed. These changes in the normal tissues naturally react on tumour cells and tissue ; the effects in the latter thus produced can be referred to, therefore, as " indirect radiation effects." They must, however, be clearly distinguished from those " indirect " effects which are assumed to be brought about by injury to the blood supply (Desjardins, 1932 ; Harvey, 1942).

The importance of the tumour-bed during treatment has been recognized by a great number of investigators (Russ, Chambers and Scott, 1921 ; Kok and Vorlaender, 1922 ; Caspari, 1922 ; Murphy, Maisin and Sturm, 1923 ; Czepa, 1924 ; Roussy, 1926 ; Ewing, 1926 ; Souttar, 1929 ; Sugiura and Cohen, 1939 ; Friedman, 1939 ; Failla, 1940 ; Ellis, 1942 ; Windeyer, 1942 ; Jolles, 1946, 1948 ; Spear, 1946 ; Windholz, 1947). Evidence was also obtained in various experiments which shows the influence of the tumour bed in the radiation response of tumours (Lasnitzki, 1947 ; Elson and Lamerton, 1949), and it was found that by taking into consideration the " stroma-reaction ", treatment methods could be devised for individual tumours in which the total dose given did not exceed 2700 r (Koller and Smithers, 1946). In the interdependent tissue structures of tumour and tumour-bed, the different reactions (cellular and intercellular, direct and indirect) are knitted together and closely integrated. It was observed that the onset, degree and rate of these reactions vary greatly in different tumours. Because the outcome of treatment depends on them, the total dose necessary for the destruction of tumours also varies within very wide limits (2700 r and 9000 r). This observation leads to the conclusion that on biological grounds the concept of a " standard tumour-lethal dose " is untenable. It is unnecessary, however, to stress that a minimal effective dose which is indispensable for the destruction of a tumour remains of paramount importance, and the enhancement of the effect of this minimal dose, by utilization of the indirect radiation effects, is here discussed.

The various reactions induced by the radiation all contribute to the resolution and eventual disappearance of the tumour, therefore the reaction-system must be considered as a whole. It is only by the analysis of all these factors and the study of the behaviour of all tissue structures, that an explanation may be found either for the inadequate response, or for the recurrence of a particular tumour. An attempt has already been made by the authors to separate the various radiation responses in order to study their relationship, interdependence and role in the

destruction of tumours (Koller and Smithers, 1946 ; Koller, 1948 ; Jolles, 1949*b*). The present paper deals with the radiation reaction induced by the " sieve-method," and summarizes the main conclusions derived from the investigation.

METHOD AND TECHNIQUE.

The irradiation is effected through a lead " sieve " with square or circular apertures, the diameter of which varies from 1·0 to 2·0 cm. This sieve or chess-board is applied tightly to the lesion to be irradiated by means of a special mould applicator built in a dental compound, which holds the sieve firmly in order to prevent the transposition of the " transparent " and " opaque " areas in successive application (Fig. 1). The technique has been fully described in a previous communication (Jolles, 1949*b*).

Apart from the direct radiation transmitted through the " opaque " lead squares (and which does not exceed 3 per cent with the thickness of lead used), the protected areas also receive a very small amount of scatter irradiation. The latter is irregularly distributed, tailing off towards the centre of the protected areas. This contingency must be taken into consideration when studying and comparing the radiation effects of the directly exposed and protected parts.

Biopsy specimens are taken simultaneously from the exposed and protected areas, preferably from the centre of these regions, and occasionally a third biopsy specimen is obtained by cutting across both the exposed and protected areas in order to follow the transitional stages of the histological changes due to radiation. The great variability of tumours in respect of the histological organization including the structure of stroma and tumour-bed, has to be kept in mind, and our observations were based only on biopsy material showing similarity in these respects.

Eighteen patients have been treated with the sieve technique, and the radiation reactions in the exposed and protected areas have been compared by the analysis of biopsy specimens. Some of these were included in preliminary reports (Jolles, 1949*a*, *b*). Two sets of experiments were devised ; in the first instance only one sieve was used throughout the treatment. In the second set, two sieves A and B, were employed ; chess-board B differs from chess-board A in that the order of transparent and opaque squares is reversed. By alternating these sieves, the previously untreated or protected areas became exposed, whilst the already treated areas became protected, thus, apart from the fractionation in time, fractionation in space is introduced. The successive application of chess-board A and B constitutes one cycle. The treatment on each chess-board might be repeated at varying intervals of time.

The procedure followed in the application of the sieve-method and the possibilities in its use are shown by Cases I and II (Fig. 2, 3, 4). They represent two extreme types in histological organization. Case I is an anaplastic (Fig. 5), Case II a differentiating squamous-cell carcinoma (Fig. 6) ; the former has well organized stroma, the latter is infiltrating by means of thin tumour cords, separated by scanty and loose stroma. The dose received either as direct (DR) or scatter and transmitted (indirect radiation, IR), by the biopsy specimens which were used for comparative analysis, is given in both cases.

Case I.—Mrs. S. J. H—, aged 80 years.

Several years' history. Proliferating ulcerated button of growth on left temple, 5 × 4 × 2 cm. fixed to underlying structures (Jolles, 1949*a*, Fig. 3).

Path. report.—" Squamous celled carcinoma of the skin. The tumour parenchyma forms infiltrating cords of varying thickness, the centre of which undergoes differentiation. There is a marked regional variation in the degree and extent of differentiation " (Fig. 5).

Alternating sieve or chess-board technique, 1 cycle with 2 series. 200 K.V., 12 mA., 0·5 mm. Cu + 1 mm. Al. H.V.L. = 1 mm. Cu. 50 cm. F.S.D. 39r/min. Applicator 7 cm. circle, chess squares 1 cm.

Dose on chess-board A = 5000 r direct (500 r × 10) in 11 days.
2 days' interval.

Dose on chess-board B = 3500 r direct (250 r × 14) in 18 days.
Overall treatment time = 32 days.

Mild reaction. Skin completely healed 44 days after end of treatment. Well to date (14 months).

Series No.	Date of biopsies.	Chess-board "sieve."	Tumour dose in areas.	
I	19.xi.48: 4 days after start of treatment	A	15.xi.48 to 26.xi.48.	
			2000 r (DR)	160 r (IR)
	28.xi.48: 13 days after start of treatment		5000 r (DR) (Fig. 9)	400 r (IR) (Fig. 10)
II	8.xii.48: 23 days after start of treatment	B	29.xi.48 to 16.xii.48	
			5000 r (DR) 160 r (IR)	400 r (IR) 1750 r (DR)
	16.xii.48: 31 days after start of treatment		5000 r (DR) 280 r (IR) (Fig. 11)	400 r (IR) 3500 r (DR) (Fig. 12)
		Total	5280 r	3900 r

Case II.—Mrs. E. R—, aged 83 years.

Eighteen months' history of ulcerated growth 5 cm. diameter on right temporal region, involving right eyebrow. Hard bean-sized right pre-auricular gland.

Path. report.—" Keratinizing squamous-celled carcinoma " (Fig. 6).

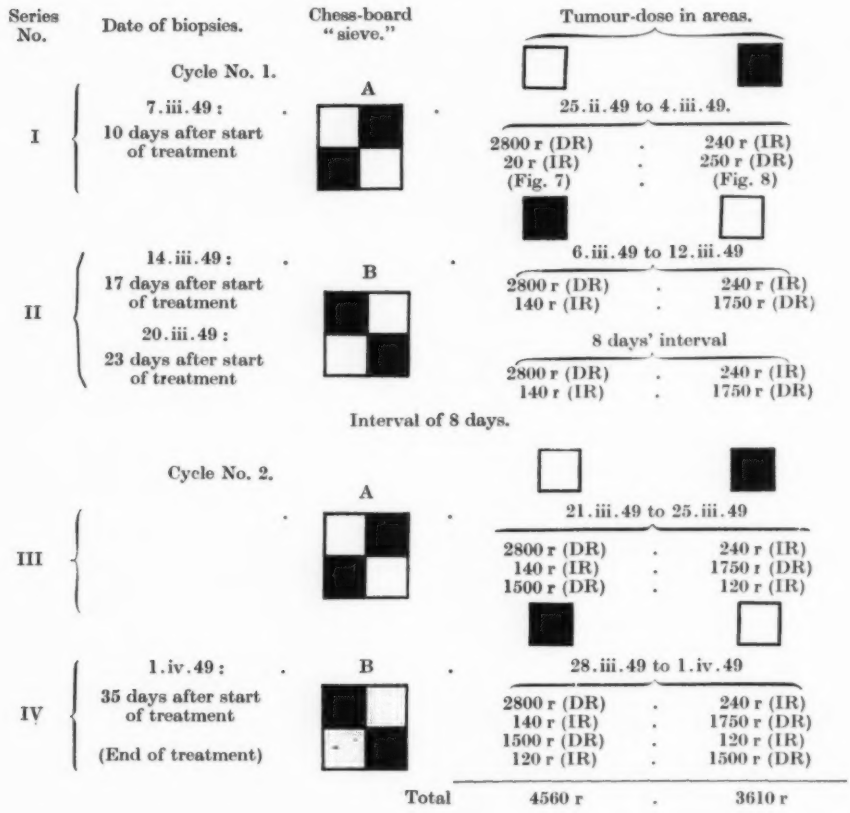
Alternating sieve or chess-board technique 2 cycles with 4 series. 200 K.V., 12 mA., 0·5 mm. Cu + 1 mm. Al. H.V.L. = 1 mm. Cu. 50 cm. F.S.D. 39r/min. Applicator 7 cm. circle, chess squares 1 cm.

Dose in 1st cycle by chess-board A = 2800 r direct (400 r × 7) in 8 days.

Chess-board B = 1750 r direct (250 r × 7) in 7 days.
Treatment free interval, 8 days.

Dose in 2nd cycle by chess-board A = 1500 r direct (300 r × 5) in 5 days.

Chess-board B = 1500 r direct (300 r × 5) in 5 days.
Overall time of treatment, 35 days.


Series No.
Date of biopsies.
Chess-board "sieve."
Tumour-dose in areas.
Cycle No. 1.
I
7.iii.49 :
10 days after start of treatment
A
25.ii.49 to 4.iii.49.
2800 r (DR) . 240 r (IR)
20 r (IR) . 250 r (DR)
(Fig. 7) . (Fig. 8)
II
14.iii.49 :
17 days after start of treatment
20.iii.49 :
23 days after start of treatment
B
6.iii.49 to 12.iii.49
2800 r (DR) . 240 r (IR)
140 r (IR) . 1750 r (DR)
8 days' interval
2800 r (DR) . 240 r (IR)
140 r (IR) . 1750 r (DR)
Interval of 8 days.
Cycle No. 2.
III
A
21.iii.49 to 25.iii.49
2800 r (DR) . 240 r (IR)
140 r (IR) . 1750 r (DR)
1500 r (DR) . 120 r (IR)
IV
1.iv.49 :
35 days after start of treatment
(End of treatment)
B
28.iii.49 to 1.iv.49
2800 r (DR) . 240 r (IR)
140 r (IR) . 1750 r (DR)
1500 r (DR) . 120 r (IR)
120 r (IR) . 1500 r (DR)
Total
4560 r . 3610 r

One month later the skin over the treated area was healed with the exception of a match-head-sized crusted spot corresponding to a " protected " square in Sieve "A" which received 2700 r in 35 days (actual dose).

The presence of an enlarged right pre-auricular gland was noticed in the patient before the commencement of treatment to the primary lesion. The gland increased in size very slightly during treatment to the lesion on the temple. One month later the gland had grown to the size of a hazel-nut. An aspiration biopsy was made and found to be positive. Radiotherapy with the alternating sieve method was decided upon, commencing on 31.v.49, and concluded on 26.vi.49. The schedule of fractionation in time and space was as follows :

6×250 r (1500 r) through sieve A. 8×250 r (2000 r) through sieve B.
6×250 r (1500*r*) sieve A. 4×250 r $+ 2 \times 500$ r (2000 r) sieve B.
(All were nominal doses—actual doses 20 per cent less.)

The gland diminished in size, and 8 months later, at the time of writing, is just palpable.

A biopsy specimen was taken from the crusted spot on the temple on 19.xii.49, eight months after the completion of treatment. The section has shown malignant tissue with no stroma; its cancerous nature was indicated by the morphology of the cells. Between 28.xii.49 and 14.i.50, 4500 r (15 × 300 r) were given to an area of 1·5 cm. diameter (140 KV., 5 mA, 0·25 mm. Cu + 1 mm. Al. filter, 15·5 cm. F.S.D.). The lesion disappeared and a moist disepithelialization over the treated area ensued. The skin healed completely 4 weeks later.

Radiation Effects in the Exposed and Protected Regions.

In a previous investigation, carried out by P. C. Koller in the Royal Cancer Hospital with the co-operation of Professor D. W. Smithers and Dr. M. Lederman, the behaviour of over 450 accessible tumours has been analysed during and after treatment, and it was established that besides the injuries in dividing cells and the great increase in cytoplasmic volume of differentiating or maturing tumour cells, the most striking change occurs in the connective tissue of the stroma and tumour-bed, which may be described as tissue "fibrosis." The connective tissue becomes more rigid in architecture, less flexible and adaptable for the development of the inflammatory reaction ("coarse" tissue fibrosis). By altering the amount of fractionated dose, and by changing the length of the interval between successive fractionated treatments, the extent and the rate of this coarse tissue-fibrosis can be modified, and its adverse effects can be reduced. There is experimental evidence which shows that a decrease in the amount of fibrosis usually increases the efficiency of the other radiation-induced responses.

Biopsy specimens taken from the exposed areas of different tumours, radiated by 2800 r–5000 r (in 8 to 11 days) directly through the sieve, have shown that fibrosis is very much less than was expected by comparison with ordinary radiation (Fig. 7, 9). Biopsies taken later from the same regions (without receiving any more additional direct radiation) have revealed the important fact that the full development of fibrosis was not only delayed, but has been curtailed. As a result of the reduced rate and extent of the fibrosis in the connective tissue of the tumour-bed, the inflammatory reaction becomes fully operative at the time when degenerative cellular changes and histological disorganization within tumour-cords begin to take place on a large scale. It is not out of place to emphasize that in tumour tissue the mitoses of adjacent or nearly adjacent cells are synchronous, hence the radiation-induced cellular injury is not additive but cumulative, and enhances greatly the development of drastic disturbances in histological organization (Koller and Smithers, 1946). This behaviour explains the great importance of a close co-ordination between stroma reaction and tumour-cell degeneration.

Another equally significant finding concerns the "indirect-radiation response" of the protected areas. After a dose in Case II of 2800 r—7 × 400 r in 8 days (all tabulated doses with the sieve technique are nominal dosed for 7 cm. circle areas, and not corrected for 1 cm. square areas, for which the doses are about 20 per cent less)—delivered to the exposed areas, it was found that the stroma reaction in these and in the adjacent protected areas is very similar (Fig. 7, 8). After 5000 r (10 × 500 r in 11 days, Case I), when only one chess-board was used, the protected areas received not more than 400 r indirect scatter and transmitted radiation, yet a slight degree of fibrosis and the initial phase of inflammatory reaction have been observed in these regions (Fig. 10). At present it is not possible

to state what is the primary cause of tissue changes in the protected areas; it may be that the small amount of scatter radiation received in ten fractions of 40 r daily can produce such alterations in the connective tissue. For that reason further experiments are in progress to study the stromal effect of such a small dose. The experimental evidence so far available indicates that tissue reaction after a dose of this order is negligible; if there is any, e.g. it does not hinder or promote wound healing (Pohle, Ritchie and Moir, 1949). We are of the opinion that the tissue changes in the protected regions are brought about by a complex mechanism in which, among others, a diffusible substance produced in the directly irradiated tissues might play a role. The reciprocal vicinity effect of irradiated tissues has been demonstrated experimentally, and it was tentatively suggested that a diffusible substance might be responsible for the latter effect apart from the reactivity of the surrounding non-irradiated tissues (Jolles, 1949*c*, 1950). Biopsy specimens taken by cutting across adjacent exposed and protected regions have shown that the extent and degree of fibrosis decreases towards the centre of the protected area, but never ceases, and suggests the action of some diffusible factor.

While there is a close similarity of tissue-reaction between exposed and protected regions, the behaviour of dividing cells shows a very clear difference in the two regions. It was observed that 24 hours after the delivery of the last

EXPLANATION OF PLATES.

FIG. 1.—Skin reaction after irradiation (11 × 500 r in 11 days). The well-defined areas showing erythema demonstrating the feasibility of applying the sieve with great accuracy. (Carcinoma of tongue, secondary glands.)

FIG. 2.—*Case II:* Squamous-cell carcinoma; 2 days before commencement of treatment.

FIG. 3.—*Case II.*—Last day of treatment.

FIG. 4.—*Case II:* 56 days after completion of treatment. The enlarged pre-auricular gland is fairly well visible.

FIG. 5.—Anaplastic squamous-celled carcinoma of forehead (Mrs. S. J. H—, Case I). × 130.

FIG. 6.—Squamous-celled carcinoma of forehead (Mrs. E. R—, Case II, Fig. 2, 3, 4). The malignant growth forms thin infiltrating cords separated by narrow connective-tissue stroma. × 130.

FIG. 7.—Section from exposed tumour area of Case II after 2800 r direct radiation (400 r × 7). Tumour cells show nuclear and cytoplasmic changes; the chromosomes of dividing cells have undergone fragmentation. × 130.

FIG. 8.—Section from the adjacent, protected area of Case II, taken at the same time as section shown in Fig. 7. The estimated radiation dose is 240 r scatter and transmitted radiation and 250 r direct radiation. Cell division is normal; stroma conditions are similar to those in Fig. 7. × 130.

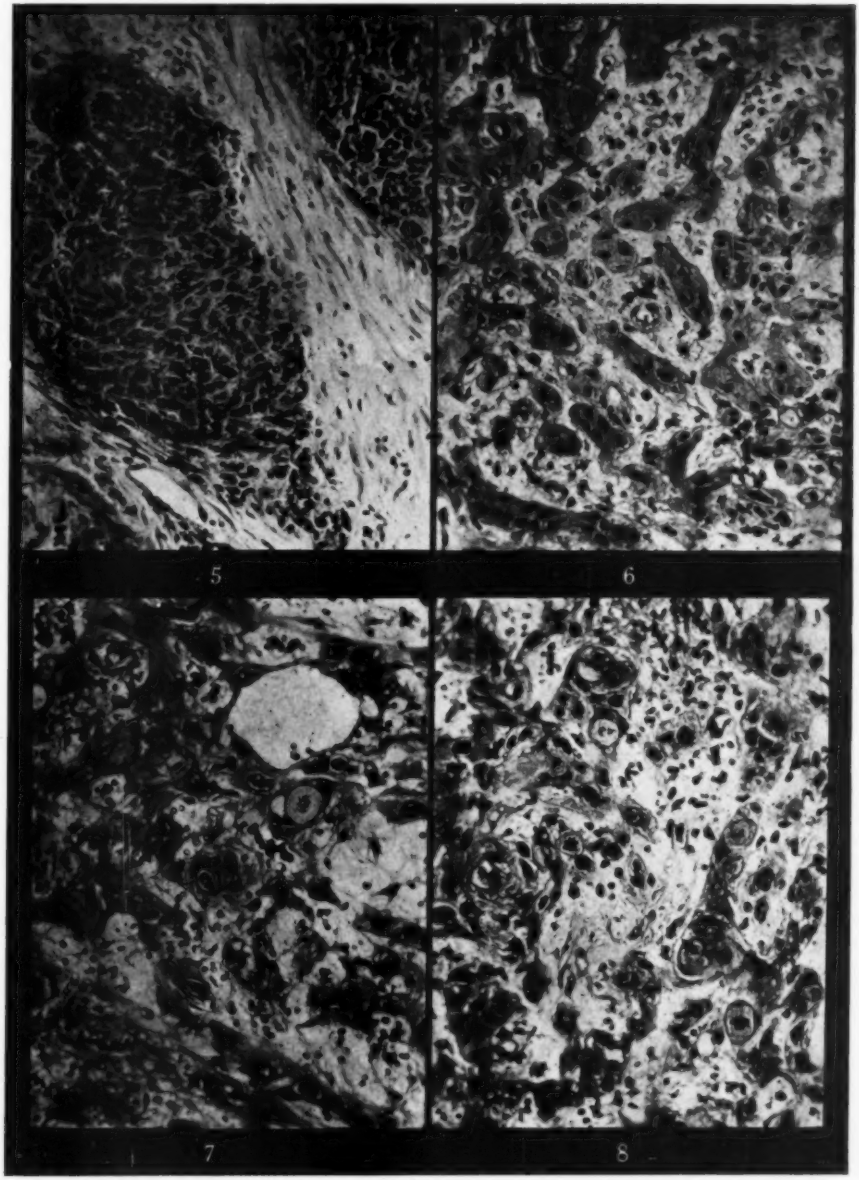
FIG. 9.—Section from the centre of exposed tumour area of Case I after 5000 r (10 × 500 r), showing fibrosis of connective tissue and the presence of lymphocytes and plasma cells in the stroma. The nuclei of tumour cells are increased in volume. × 130

FIG. 10.—Section from the centre of protected area of the same tumour (Case I), taken at the same time as section shown in Fig. 9. Radiation dose is 400 r. The histological picture is similar to that in Fig. 9 except that the fibrosis is somewhat less and the inflammatory reaction shows an earlier phase. × 130.

FIG. 11.—Section from the exposed area 18 days after 5000 r direct radiation. It received 280 r scatter radiation during this period. The connective-tissue stroma is loose, and there is little fibrosis. Polymorphonuclear leucocytes are invading into the tumour cord, around which there is an accumulation of lymphocytes, macrophages and plasma cells, indicating an inflammatory process. All tumour cells in the cord have undergone degeneration. × 130.

FIG. 12.—Section from the area adjacent to that shown in Fig. 11. It received 3500 r direct and 400 r scatter radiation. The radiation-induced changes in the tumour cord are similar to those seen in Fig. 11. Connective tissue of the stroma is loose, and displays a somewhat smaller inflammatory reaction than seen in Fig. 11. × 130.

Jolles and Koller.



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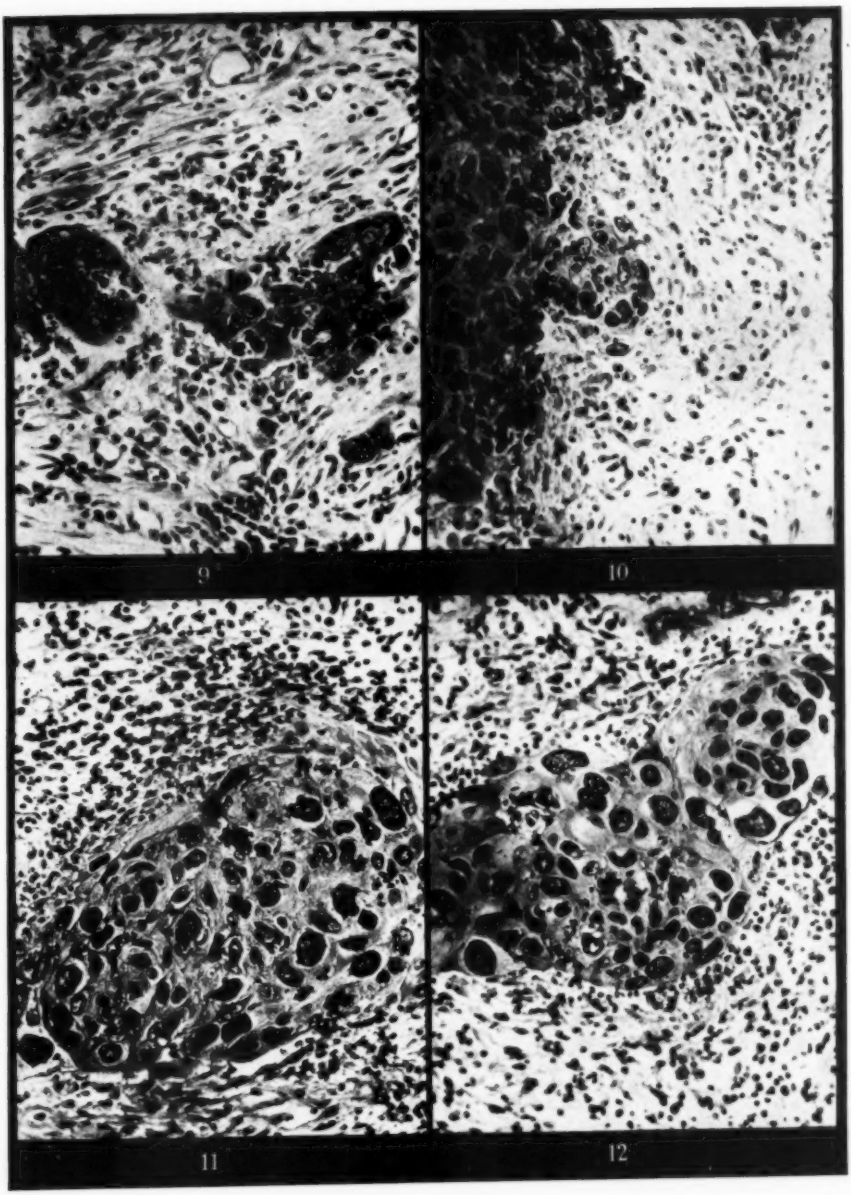

Jolles and Koller.

fractionated dose, giving a total of 2800 r (Case II), all the cells in mitosis exhibit chromosome fragmentation, i.e. direct radiation injury; on the other hand, in the adjacent protected regions (240 r scatter radiation) no abnormalities were seen in dividing cells (Fig. 7, 8). The absence of chromosome fragmentation is another proof that the alteration seen in the protected areas, such as the increase in the size of tumour cells, and the slight degree of fibrosis and inflammatory reaction, must be considered as an indirect reaction to radiation.

Our investigation has shown that the rate and degree of the indirect radiation reaction depends on the histological organization of stroma, and on the dose. On account of the differences in the histological architecture of tumours, the minimum effective dose varies from tumour to tumour. We found that in tumours in which the connective tissue is abundant the minimum effective dose is smaller than in tumours with scanty stroma. This is illustrated in Case II, in which the dosage used (2700 r actual dose in 35 days) did not reach the critical minimum level necessary to produce a reaction of sufficient strength and efficiency in the scanty connective tissue of the " protected " tumour areas. On the other hand, the use of the sieve-technique has permitted the subsequent treatment of the residual tumour with a relatively high but adequate dose (4500 r actual dose in 18 days), because the histological texture of the skin, owing to the small size of the areas directly exposed to irradiation during the initial treatment has been left unimpaired, and could safely be subjected to such a high dose in the second treatment.

The complexity of the various cellular and tissue interactions increases when further radiation is carried out through another " chess-board or sieve " in which the position of transparent and opaque areas is reversed. By increasing the

TABLE I.—*The Effect of Radiation on Cells and Tissues.*

Cells and tissues.	*Intracellular effects.*		*Intercellular or tissue effects.*
	Dividing cells.	Interphase and differentiated cells.	
Malignant	Chromosome fragmentation Chromosome bridges Nuclear deficiency " Pycnosis "—cell death	Increase in cell size: "over-differentiation" Disturbance in enzyme activity	Integrated effects— Synchronous division of injured cells. Regional breakdown and tissue necrosis. Alteration in histological organization. Undefined " physiological " effects with changes in surface tension, in pH, in osmotic pressure, etc.
Normal (tumour bed and stroma)	Same as above. Difference is only in degree and rate, not in quality of the induced effects		Integrated effects— *Stroma-reaction:* (i) *directly induced.* (local defence-reaction). Introduction or increase of the process of " inflammation." Invasion of lymphocytes into connective tissue. Plasma-cell differentiation. Fibrosis; excessive deposition of collaeng. (ii) *Indirectly induced.* Same as above, produced by "diffusible substances" in adjacent, non-irradiated areas.

number of cycles, i.e. by alternating the chess-boards twice, three or four times the rate and intensity of the tissue reactions can be greatly affected. We found in the cases investigated that at the completion of treatment the excessive tissue fibrosis was absent, and that the tumour-bed and stroma reactions are uniform in degrees and type in regions which received significantly different doses, such as 5280 r and 3900 r in Case I (Fig. 11, 12) and 4560 r and 3610 r in Case II respectively. Furthermore it was observed that the histological alterations in the tumour-bed have brought about an environment which is particularly well suited for the development and maintenance of the " inflammatory " reaction, induced by the local defence system of the body under the influence of radiation.

The application of the sieve-method has enabled us to distinguish clearly between the various reactions in normal and malignant tissues, to study their integration and to estimate their relative importance in the radiation response of tumours (Table I, Jolles, 1949*b*). It became firmly established in the course of our investigation that the radiation reactions in the connective tissue of the tumour-bed and the stroma play a very important role in the destruction of tumours. We found that while the connective tissue needs protection from excessive radiation, it requires a certain quantity of radiation and a specific distribution in time and space of this quantity in order to elaborate the radiation-induced reaction. We have seen that on the one hand the sieve method greatly favours the induction and maintenance of this reaction, and on the other hand, it does not impair the basic structure of the histological organization of normal tissues.

DISCUSSION.

In the present-day practice of radiotherapy of squamous epitheliomata, the most important guiding principle seems to be " the delivery of the highest dose possible within the shortest time," the effective dose being that which the skin can tolerate, and the aim is to suppress mitosis and induce " differentiation " in the tumour. The biological aspect which underlies the above concept takes into consideration changes or reactions within *individual* tumour cells, but disregards the tissue environment with its great variability (Scarff, 1948). This drastic simplification of the biological complexity of tumour behaviour is responsible for the establishment of some uniformity of treatment for the most diverse neoplastic growths.

The cytological study of the radiation reactions of tumours during and after treatment has shown that by the daily repetition of fractionated doses of 500–600 r, the reactions are accelerated and intensified to such an extent and in such a manner that the interaction of the various responses becomes greatly disorganized (Koller and Smithers, 1946 ; Koller, 1948). Extensive coarse fibrosis is of common occurrence, and may be produced in the stroma early in treatment. This not only prevents the development of an inflammatory process in the connective tissue of the tumour-bed, but a protective barrier is formed which shelters islands of tumour cells from further radiation damage. It has been found that the daily repetition of the fractionated dose more often than not interferes unfavourably with the tumour-bed and stroma reactions and greatly hinders their efficiency.

Since the fact that reactions of the connective tissue play an important role in the destruction of tumours has been disregarded in order to achieve " cures,"

the highest dose which the skin can tolerate is used. The stroma and tumour-bed reactions, however, being too obvious to disregard completely, are referred to, but only as concomitant, " independent " events, which have no, or too little, bearing on the radiation response of the malignant growth itself. The most cursory histological study of tumours under radiation reveals the fact that the destruction of all malignant cells of the tumour parenchyma rarely, if ever, can be achieved by the " direct " action of a therapeutic dose. It seems, therefore, necessary and logical not only to admit the existence of an " indirect " effect, but to accept all the implications of the generic statement that the purpose of radiation is to kill a variable but significant proportion of active tumour cells, and at the same time to assist the local body defence system by inducing the various responses in the tumour-bed and stroma.

It also follows that any view based on changes induced by radiation in some and not all tumour-tissue components is not comprehensive, and that the conclusions drawn from the study of cell behaviour alone are doubtful. On that account the "cell count method" of Glücksmann and co-workers (Glücksmann, 1941, 1948; Glücksmann and Spear, 1945; Glücksmann and Way, 1948), which *per se* represents a contribution to radiobiological knowledge, is liable to be misleading when claims are being attached to it, such as that it provides an explanation for the variation of radiation response of tumours, predicts the outcome of treatment, and indicates the most appropriate treatment method, whether surgical or radiological, for a given case. The cell count method is biased by subjective selection and interpretation; it disregards the great regional heterogeneity shown by tumours both in growth rate and histological organization, and the fact that a malignant growth is composed of tumour and stroma, which form one closely integrated unit. It is more than obvious that for that reason the application of the cell-count method to biopsy specimens taken after a " test dose " of radiation in order to decide whether a tumour be submitted for surgery or radiotherapy is of doubtful value (Glücksmann and Way, 1948).

It has been stated by Spear (1946) that " radiation affects any given cell of a complex tissue in at least two ways, first by direct action on the cell, and secondly by injuring neighbouring tissues, upon the healthy functioning of which the cell depends." Our investigation leads us to a similar conclusion that the direct cellular effects, e.g. fragmentation of chromosomes, represent only the initial phase, and that the most important factors responsible for the disorganization, regression and re-absorption of tumours during and after irradiation are the various, closely integrated and related reactions which are produced by the radiation in the connective tissue of tumour-bed and tumour stroma.

The present investigation, in which the alternating chess-board method was employed, has shown the role of the connective-tissue reaction in the destruction of tumours and the way by which this reaction can be favourably influenced and modified. We have found that the fractionation of dose in time as well as in space, as carried out by the sieve, induces specific changes in cells and tissues. The intensity and rate of these changes affects the whole architecture of stroma and tumour-bed as well as that of the tumour. Our investigation has also shown that when the sieve is used sufficient response can be produced in the local defence system of connective tissue, and can be maintained by a much smaller dose than usually employed. The protection of connective tissue by the sieve enables

us in cases where the tumour is radio-resistant, either by its nature or by its environment, to deliver a higher total tumour-dose without the danger of destroying vital structures of the tumour-bed. It is our intention to investigate the potentialities of this new procedure by applying the sieve method for the treatment of radio-resistant and deep-seated tumours, especially metastatic deposits in lymph nodes. The sieve or chess-board method is not designed for the treatment of small superficial epitheliomata, which can be dealt with successfully by more expeditious techniques at present in use. It offers a standby in difficult extensive cases in which a real risk of necrosis has to be faced in order to eradicate the tumour, or in cases in which radiotherapy registers failures more often than cures.

SUMMARY.

The radiation reaction in 18 cases of accessible tumours treated by the sieve-method has been analysed. Treatment has been carried out through a lead " sieve " or " chess-board " with square or circular apertures of 1·0–2·0 cm. in diameter. The tumour is divided into a number of " exposed " and " protected " areas, the former receiving direct, the latter receiving only a small amount of scatter and transmitted radiation. Two chess-boards or sieves, which differ in the order of the transparent (exposed) and opaque (protected) squares, can be used during treatment.

Irradiation induces injuries in the tumour cells and alterations in the connective tissue of the tumour-bed and stroma. The latter consists of fibrosis and a reaction closely resembling inflammation. The radiation reaction in the connective tissue plays a very important role in the destruction and reabsorption of tumours.

In tumours irradiated through the sieve it was observed that tissue-reaction is reduced in the directly " exposed " regions, but at the same time, fibrosis and inflammatory reactions are induced in the " protected " regions. No cellular injuries, due to direct radiation such as chromosome fragmentation, were seen in cells of the " protected " areas.

By using two sieves which differ in the order of the " transparent " and " opaque " areas, the reaction of the tumour-bed and stroma in the presence of abundant connective tissue becomes uniform throughout the tumour, irrespective of the different doses which regions may have received during treatment.

Analysis of the r[illegible]tion reaction in tumours treated by the sieve has shown that fractionation of [illegible]se in time as well as in space induces injuries and specific alterations in the i[illegible]diated area, the rate and type of which greatly enhances tumour destruction.

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EFFECTS OF PREGNANCY AND LACTATION ON THE GROWTH OF A TRANSPLANTABLE SPINDLE CELL SARCOMA IN CBA MICE.

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HAALAND (1907), Ehrlich (1908) and Loeb (1917) observed that in mice pregnancy was an unfavourable factor for the growth of transplanted tumours. Kross (1921) concluded that pregnancy had no effect on a transplantable rat carcinoma.

Haddow (1938) noted a decline in growth rate of spontaneous mammary carcinomas in mice during lactation, in the same way that Dobrovolskaia-Zavadskaia (1930) had reported partial regression of similar tumours during that period. Cuénot and Mercier (1909) recorded regression of Borrel's mouse tumour " B " during lactation, and Emge and Wulff (1934) found depression in growth curves of small fibromas and adenofibromas in the rat in the same period.

MATERIAL AND METHODS.

In the present experiment sexually mature pure CBA female mice, bred in the laboratory and segregated from males for 2 months, were used. Three females and one young male mouse were kept together for 3 days in each box, during which time the females were examined every morning and evening for copulatory plugs. On the 4th day the males were removed from the boxes and 39 females which had shown plugs were placed in separate boxes, these constituting the experimental group. Forty-three unmated female mice of similar average age, and wherever possible litter mates of the experimental mice, made the control group.

On the day of separation, each of the mice received a subcutaneous graft, roughly 2 mm. $\times$ 2 mm., in the right flank, from a 25-day-old transplantable methylcholanthrene-induced spindle-cell sarcoma growing in CBA mice. This tumour retained the original histological appearance and characters of steady and uniform growth through successive generations, and was chosen because its rapid growth was considered an advantage in view of the shortness of the pregnancy and lactation periods in mice.

From the 10th day following grafting the tumours were measured every 4th or 5th day in 2 diameters (length and breadth) until the end of lactation, i.e. until 19 days following parturition. Two indices were calculated to assess the rates of growth of the tumours ; the index

$$\frac{a' \times b' \text{ (area on the day of parturition)}}{a \times b \text{ (area on the 10th day of grafting)}}$$

expresses the rate of growth during

pregnancy and the index $\frac{a'' \times b'' \text{ (area at the end of lactation)}}{a' \times b' \text{ (area on the day of parturition)}}$ that during lactation. These indices have been accepted as proportional to the results obtained by using Blum's (1943) formula, assuming the shape of the tumour to be an ellipse (Bonser, 1948). The course of tumour growth is also represented in Fig. 1 by curves obtained by plotting the average of the sums of 2 diameters ($l + b$) against time, as described by Haddow (1938).

Because of the difficulty in obtaining a large number of pregnant mice at one time, the experiment had to be performed on 6 batches of mice, 25-day-old tumours of successive generations being used for grafting. When the tumours

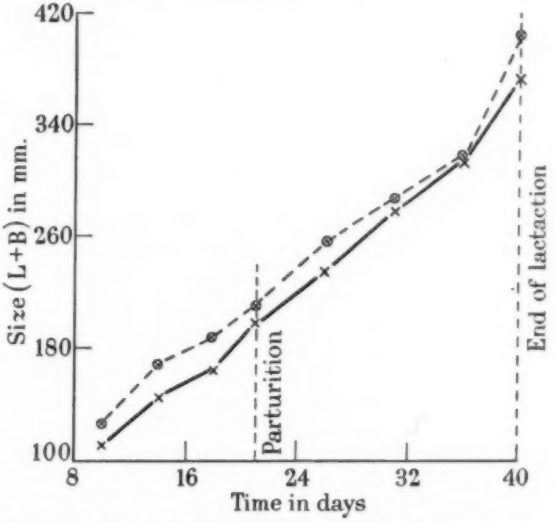

FIG. 1.—Rate of tumour growth in experimental and control mice.
×——× Experimental.
⊗ - - - - ⊗ Control.

were not measured on the exact day of parturition or end of lactation, the measurements recorded on the day immediately preceding or following were employed. Four experimental and 2 control mice had tumours too small to measure on the 10th day of grafting, and their rates of growth during pregnancy could not be determined. Three mice (2 experimental and 1 control) grew more than one tumour close to one another. These are not included in the results. Five of the experimental mice killed their litters the day they were born. In all cases other CBA litters about a week old were substituted. The mice suckled them and the young grew normally.

RESULTS.

In Tables I and II the growth rates of all tumours are recorded, and, employing an arbitrary classification into completely regressing, partially regressing, slowly

growing, moderately growing, and rapidly growing tumours, arranged in five groups.

All but 2 of the tumours in the control mice grew steadily from their first appearance, and their course was represented by an approximately straight line (Fig. 1). One of the tumours regressed completely during the week following parturition, whilst the second began to regress during the same period and continued to regress to the end of the period of lactation.

TABLE I.—*Rate of Growth During Pregnancy (Summary of Indices).*

$\frac{a' \times b'}{a \times b}$	$\frac{a' \times b'}{a \times b}$		Rate of growth.
	Experimental.	Control.	
Disappeared	0	0	Complete regression.
<1	0	0	Partial regression.
1·0–1·99	6	9	Slowly growing.
2·0–2·99	10	10	
3·0–3·99	3	8	Moderately growing.
4·0–4·99	1	6	
5·0–5·99	5	2	
6·0–6·99	2	1	Rapidly growing.
7·0–7·99	4	1	
>8	2	3	
Total mice	33	40	

TABLE II.—*Growth During Lacation (Summary of Indices).*

$\frac{a'' \times b''}{a' \times b'}$	$\frac{a'' \times b''}{a' \times b'}$		Rate of growth.
	Experimental.	Control.	
Disappeared	0	1	Complete regression.
<1	0	1	Partial regression.
1·0–1·99	3	3	Slowly growing.
2·0–2·99	11	6	
3·0–3·99	6	13	Moderately growing.
4·0–4·99	7	11	
5·0–5·99	4	4	
6·0–6·99	5	2	Rapidly growing.
7·0–7·99	0	0	
>8	1	1	
Total mice	37	42	

Effect of pregnancy.

From Table I it is seen that there is no difference in growth rate of the transplanted tumours in the 2 series. The slightly slower growth rate in the experimental mice among the tumours classed as "moderately growing" is of no statistical significance.

TABLE III.—*Percentage Incidence of Slowly, Moderately and Rapidly Growing Tumours During Pregnancy and Lactation.*

Rate of growth.	Pregnancy.		Lactation.	
	Experimental. %	Control. %	Experimental. %	Control. %
Slowly growing	48·49	47·5	37·84	22·5
Moderately growing	27·27	40·0	45·95	70·0
Rapidly growing	24·24	12·5	16·21	7·5

Effect of lactation.

From Tables II and III it is seen that the tumours in the experimental series tended to grow less rapidly than in controls, especially when consideration is given to the moderately growing group. Here the difference between the two series is approximately 24 per cent, which is more than twice the standard error ± (10·93). This degree of significance would suggest a retarding effect due to lactation, but is not quite convincing, since the observation relates to a small group of animals.

When, however, the growth curves in both groups of mice are considered over the whole period of observation (i.e. both during pregnancy and lactation) there is no flattening of the curves at any period. In other words there is no alteration in the intrinsic growth rate of the tumour.

DISCUSSION.

There was no diminution in the intrinsic growth rate of a transplantable homologous methylcholanthrene-induced spindle-cell sarcoma in CBA mice during pregnancy, and no diminution in growth rate compared with that in control mice. This is in contrast with the observation of other authors quoted above. In lactation, however, although there was no alteration in the intrinsic growth rate, there was a slight lag in comparison with a control series.

The possible factors concerned in any alteration of the growth rate of tumours during lactation are (1) hormonal and (2) nutritional. It seems unlikely that the effect of lactation observed in this experiment could be attributed to a hormonal influence, because the anterior pituitary hormone, prolactin, which would be active in the period of lactation, was noted to have no effect on tumour growth (Bischoff and Maxwell, 1936). Besides, Gardner (1942) reported persistence and growth of spontaneous mammary tumours in hypophysectomized mice.

As far as nutritional factors are concerned, it is to be noted that all the mice littered, the litters being of average size and healthy. All but five of them suckled their young. During pregnancy the tumours were small, and could obtain the necessary nutriment for growth. During lactation they grew bigger with an increase in their nutritional requirement, which does not seem to have been

available in the experimental mice to the same extent as in the controls. This would be in keeping with the views expressed by Slye (1920) and Kross (1921), that since reproduction and cancer are both growth processes, there is a forced division of the available nutriment between the tumour and the offspring.

SUMMARY.

Pregnancy and lactation did not alter the intrinsic growth rate of a transplantable methylcholanthrene-induced spindle-cell sarcoma in 79 CBA mice.

Tumours grew relatively less rapidly in lactating mice than in non-lactating controls.

It is suggested that following pregnancy and parturition all the necessary nutriment for tumour growth was not available.

I am grateful to Professor R. D. Passey for permitting me to carry out this experiment in his department. I should like to record my thanks to the late Dr. J. Flaks for his suggestions and for providing the spindle-cell sarcoma, and to Dr. G. M. Bonser for her guidance.

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BLOOD AMINO NITROGEN IN TUMOUR-BEARING MICE.

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THERE is an extensive literature on the chemical abnormalities of the blood of cancerous patients and tumour-bearing animals. However, very little work on disturbances in the concentration of amino acids in the blood of tumour-bearing hosts has been published. Goldfeder (1934), who studied the different fractions of non-protein nitrogen in man, observed a rise in the level of the amino acid nitrogen in the blood of cancerous patients. In most cases this increase was slight, but in a few instances of cancer of the breast his figures were about double the normal value. Malowan (1932) also found blood changes of the same low order, though Becher and Herrmann (1932) stated that they were unable to find any significant change that could be attributed to the presence of the tumour.

All the above workers used Danielson's modification of Folin's well-known colorimetric method for blood amino acids. A study, by a more specific quantitative method, of the α-amino nitrogen in the blood of mice bearing experimentally transplanted tumours is reported in this paper.

MATERIALS AND METHODS.

Blood amino acid method.—Determinations of the amino acid nitrogen in the blood were made utilizing the principle described by Van Slyke, MacFadyen and Hamilton (1941), which is based on the fact that α-amino acids when boiled with excess of ninhydrin at pH's between 1 and 5 evolve the CO_2 of their free carboxyl groups quantitatively in a few minutes. The estimations were made by a modification of the original method devised by my colleague, Dr. R. W. R. Baker, of the Chemical Pathology Department (Baker, 1948). The all-glass apparatus used is shown in Fig. 1. The method is specific for nitrogen in the form of free α-amino groups adjacent to terminal carboxyl groups. It therefore gives an estimate of the nitrogen of free amino acids and of the terminal groups of peptides.

Tumours used.—The mice used carried the transplantable carcinoma M 2146 of the Imperial Cancer Research Fund. This cancer was originally a tar tumour of a highly malignant character. It was maintained by regular transplantation every fortnight; the animals were of both sexes, and were used only as long as they were free from infection and ulceration.

Diets of animals.—For the greater part of the work all the mice were kept on a constant diet of "Calf Cubes," prepared by the Associated Flour Millers, Bankside House, Leadenhall Street, London, E.C.1. In some of the later experiments mice were placed for short periods on a diet deficient in proteins and

amino acids, the composition of which is as follows : Starch 85·8 g., nut oil 12·5 g., dried yeast 1·3 g., salts 0·4 g., water 9·5 g.

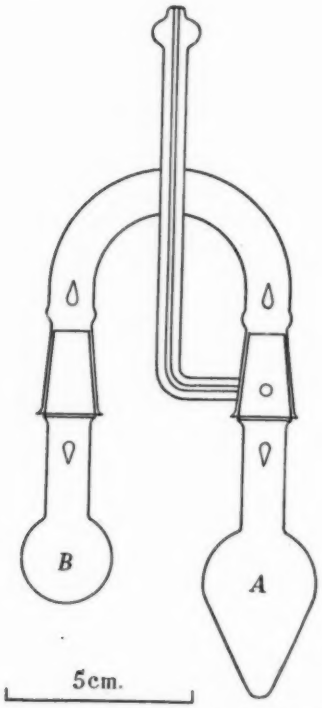

FIG. 1.—Modified apparatus for the determination of α-amino nitrogen.

RESULTS.

Blood α-amino nitrogen in normal mice.

Three batches of healthy mice, each 8 to 12 in number, of either sex, were used to establish the normal level of blood α-amino nitrogen in animals maintained on the above " Calf Cube " diet. The results are shown in Table I.

TABLE I.—*The Mean Blood α-Amino Nitrogen in Normal Mice.*

Number of mice in batch.	Mean blood α-amino nitrogen (mg. per cent).
10	4·67
12	4·82
8	4·78

From these determinations it was found that the mean and standard error of the concentration of the α-amino nitrogen in the blood of mice used under the

standard conditions of diet and care employed in the subsequent experiments was 4·76 ± 0·13 mg. per cent.

Blood α-amino nitrogen in tumour-bearing mice.

In the mice used in these experiments, the tumour was allowed to grow for various periods before the animal was killed by decapitation and blood was collected from the divided vessels. The weights of the animal just before death and of the enucleated tumour were determined. The results which show the relationship between the tumour weight as a percentage of body weight and the blood α-amino nitrogen determinations are shown in Table II.

TABLE II.—*Percentage Tumour Weights and Blood α-Amino Nitrogen Values in Tumour-bearing Mice at Various Intervals after Implantation.*

Age of tumour (days).	Animal weight (g.).	Tumour weight as percentage of body weight.	Blood α-amino nitrogen (mg. per cent).
17	26	1·6	5·8
21	25	3·4	5·8
23	18	3·6	5·0
28	38	4·0	7·6
22	25	4·9	5·2
16	29	6·8	5·7
23	23	7·0	6·0
20	28	7·5	6·2
16	37	8·1	6·5
15	28	8·5	6·2
20	27	10·0	6·2
16	27	10·5	7·0
16	34	11·2	7·1
15	28	12·0	7·2
21	25	12·8	8·5
15	27	13·0	7·6
16	41	14·0	10·0
16	43	14·2	7·9
15	37	15·3	7·0
19	30	15·8	7·8
20	28	16·0	8·4
19	28	16·0	7·7
21	32	16·3	6·2
21	28	18·0	8·3
19	31	18·2	8·0
22	33	20·0	8·3
21	29	20·4	8·2
21	31	21·9	7·7
17	33	23·0	8·5
20	35	25·9	8·2
22	33	28·0	9·1
21	27	29·3	8·8
21	35	30·0	8·9

The average value for the blood α-amino nitrogen level in these tumour-bearing mice was found to be 7·35 mg. per cent. In further experiments similar values were again found; the average for the whole series of 55 mice with tumours of various sizes was 7·40 mg. per cent.

From Table II it seems likely that there is a significant positive correlation between the percentage weight of the tumour and the blood α-amino nitrogen level. For, while tumours less than 5 per cent of the body weight appear to produce hardly any increase, those of 10 per cent and over are accompanied by a definite rise in the α-amino nitrogen values. This relation is presented graphically in Fig. 2.

These observations show that the growth of the transplantable carcinoma M 2146 is accompanied by an elevation in the blood α-amino nitrogen. While the mean value in normal mice was 4·76 mg. per cent, that in the tumour-bearing

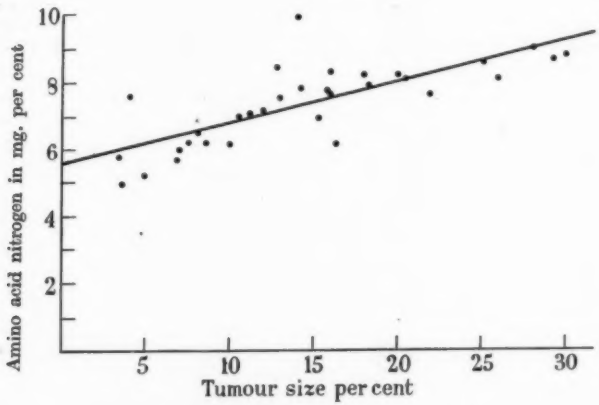

FIG. 2.—Relation of tumour size to amino acid nitrogen per cent in blood. Correlation coefficient = 0·7530 ± ·174. Amino acid nitrogen = 0·1213 T.S. +5·63.

mice was 7·40 mg. per cent. It is clear also that this elevation did not take place suddenly, but that it was intimately related to the proportionate size of the tumour, and this association was further demonstrated by the finding of a coefficient of correlation between the two which had the highly significant value of 0·753 ± 0·176. The corresponding regression equation between these variables is: α-amino nitrogen values = 0·1213 tumour percentage size + 5·63. These changes in the level of the blood α-amino nitrogen probably account for the increase in the blood non-protein nitrogen in tumour-bearing animals found by other authors (Greenstein, 1947).

Blood α-amino nitrogen in mice with regressing tumours.

In about 10 per cent of the mice used in these experiments the tumour, after having grown and reached a definite size, regressed completely. It seemed of interest to estimate the blood amino acid concentrations in these animals. The findings for 10 such mice are shown in Table III.

TABLE III.—*Blood α-Amino Nitrogen Concentrations in Mice in which a Tumour has Grown and Regressed.*

Days after inoculation.	Weight of animal (g.).	Blood α-amino nitrogen (mg. per cent).
24	24	4·4
28	31	6·3
36	35	5·7
45	30	4·8
45	28	4·2
45	32	5·0
48	35	4·8
55	40	5·0
60	42	4·1
60	37	4·3
	Mean	4·86

After regression of the tumour, the α-amino nitrogen level of the blood returns almost exactly to its former normal value. A similar tendency to return was observed in 2 mice whose superficial, but growing, tumours were successfully enucleated surgically. The values of their blood α-amino nitrogen five days subsequently were 6·0 and 5·9 mg. per cent.

The effect of protein-deficient diets on the blood α-amino nitrogen in tumour-bearing mice.

A study was made of the changes in the blood α-amino nitrogen concentrations in normal and tumour-bearing mice when both had been placed for short periods on a diet that was deficient in protein. The composition of this diet has already been given. The animals consumed this food freely for the first two days, but seemed to lose appetite during the remainder of the experimental five-day period. The loss of weight, which had become severe by the fifth day, together with the blood α-amino nitrogen values, are shown in Table IV.

With the introduction of the deficient diet there was a marked loss of weight in both groups of mice ; the tumours, however, continued to increase in size even at the expense of the host, which was clearly in negative nitrogen balance. The tissues of the host presumably break down to supply the amino-acid needs of the growing parasitic tumour. The early fall in the blood α-amino nitrogen, before any marked loss of weight occurred, seems to indicate the extent to which the concentration of these substances in the blood is dependent upon the state of alimentation. In the normal mice the persistence of the α-amino nitrogen level at about 2 mg. per cent throughout most of the experiment seems to show that this represents the basal level for this group of substances, below which further fall is resisted. In the tumour-bearing group, on the other hand, the concentration of the α-amino nitrogen fell rapidly to between 4 and 5 mg. per cent, and continued at this level for the subsequent three days. The similarity of the fall to that found for the control group would seem to show that whatever metabolic change was responsible for the characteristically elevated blood α-amino nitrogen level was independent of the state of alimentation.

TABLE IV.—*Loss of Weight and Blood α-Amino Nitrogen Concentrations in Control and Tumour-bearing Mice Maintained for Varying Periods on Protein Deficient Diets.*

Original weight (g.).	Loss of weight (g.).	Duration of diet (days).	Blood α-amino nitrogen (mg. per cent).
	Controls.		
30·1	1·2	2	2·1
26·5	1·5	2	1·8
27·4	1·6	2	2·2
29·5	3·7	4	2·0
23·5	4·1	4	1·7
24·6	5·1	5	2·2
25·5	5·6	5	1·9
30·8	5·4	5	2·1
	Tumour-bearing.		
25·8	1·4	2	4·8
25·5	1·2	2	5·0
28·4	2·3	2	4·8
29·2	4·4	4	4·7
27·4	5·9	4	4·0
30·6	6·1	5	5·1
31·8	6·8	5	4·7
32·4	4·9	5	5·4

When the surviving mice from each batch were replaced upon the normal diet they put on weight, and the concentration of their blood α-amino nitrogen soon reverted to the earlier levels. Since the protein-deficient diet resulted in equal falls in both groups of mice, the source of the α-amino nitrogen which contributes to the high concentration of these substances in the blood of tumour-bearing animals would appear to be the tumour itself.

DISCUSSION.

The method of analysis used for this work records specifically all the α-amino groups present in free amino acids, together with those that occur as terminal groups in the non-heat-precipitable polypeptides. The material rise in the values found for these groups in the blood of tumour-bearing mice, roughly about 3 mg. per cent, may thus be in either non-protein nitrogen fraction. There is little evidence that the free α-amino acids are increased in tumorous animals, but observations on rats with a transplantable hepatoma have shown that the second fraction may on average be raised seven or eight fold (Winzler and Burk, 1944). As with the findings here, the raised value reverts to its former normal level should the tumour regress or be resected surgically. It seems probable, therefore, that the rise in the α-amino nitrogen in the blood of our tumour-bearing mice is mostly, if not wholly, attributable to the presence of these polypeptide components.

Normally, the amino acids of the blood are derived from two sources : (i) those that are absorbed from the intestines and have escaped deamination or synthesis

into protein in the liver; and (ii) those liberated by the breakdown of tissues and not yet metabolized. Although it is known that the concentration of amino acids in the blood is raised during the digestion of protein (Bolton and Wright, 1937), the elevation found here in the tumour-bearing animals clearly cannot be accounted for in this way. The elevation continues almost unabated when the animals are placed on a diet wholly deficient in protein. It seems, therefore, that the increase in α-amino nitrogen must be attributable to one or other of the following factors: a local increase in the production of amino acids or of polypeptides by the tumour itself, either through the degeneration of its cells or the hydrolysis of normal body proteins brought to it by the circulation; or to some hepatic dysfunction that is present in tumour-bearing animals which interferes with the normal disposal of these substances, and thus permits them to accumulate in the blood. This latter possibility will be considered first.

Fujiwara (1929), Weil (1935), Greenstein, Jenrette, Mider and White (1941), and El Mehairy (1949) found little inhibition of liver arginase in tumorous animals. Greenstein (1947) also noted no dimunition in cystine desulphurase activity in the same organ. Arginase and cystine desulphurase are two of the main liver enzymes concerned with the intermediary metabolism of amino acids, and the study of their behaviour provides valuable information upon disturbances in the hepatic enzyme pattern generally. Robertson and Kahler (1942) and Lan (1944) have also failed to observe any decrease in the enzymic activity of livers in animals bearing tumours. From this accumulated evidence the conclusion that the enzymes concerned with the intermediary metabolism of amino acids in the liver are only slightly, if at all, depressed by the growth of the tumour seems justified, provided that this organ is not the seat of primary or secondary neoplasia. Such small depression of liver function as some authors have reported seems insufficient to account for the rise in the values of α-amino nitrogen by over 50 per cent. This conclusion as to hepatic enzymes was supported by the results of histological study of livers from tumour-bearing mice, for numerous sections from such livers revealed no sign of abnormality.

The experiments in which mice were maintained for several days on a diet deficient in nitrogen showed clearly that such treatment brought the blood α-amino nitrogen down to a minimum value below which no further fall took place. In the tumour-bearing group of mice in this experiment the concentration of α-amino nitrogen in the blood was consistently higher than in the control animals; this difference was almost constant for the first days of the deficient diet to the end of that experimental period. This elevation in the tumour-bearing animals would thus seem to be attributable to the participation of the tumour cells themselves.

Further evidence as to the source of the extra α-amino nitrogen in tumour-bearing mice came from the study of animals with regressing tumours. With the complete disappearance of the tumour the level fell back to within the normal range. That this regression was responsible for the decline in the blood α-amino nitrogen level is corroborated by the finding of a similar reversion in the 2 mice in which progressively growing tumours were resected surgically. It seems, therefore, highly probable that the increase in the concentration of the α-amino nitrogen in the blood is due to the release of peptides or α-amino acids from the tumour itself, possibly as a result of autolysis of dead cells. Such breakdown products probably form in the interior of the tumour mass, where degeneration

and necrosis are known to proceed, and leaking thence into the surrounding blood and lymph vessels, gain access to the general circulation.

SUMMARY.

The concentration of α-amino nitrogen in the blood of tumour-bearing mice was found to increase much above that found in comparable normal animals. This increase was correlated with the proportionate size of the tumour. Tumours of less than 5 per cent of the total body weight were not accompanied by any significant change in the α-amino nitrogen concentration in the blood.

The tumour tissue itself is believed to be the source of extra α-amino nitrogen in the blood.

I wish to record my indebtedness to Professor G. Payling Wright for his advice and criticism. My thanks are also due to Dr. R. W. R. Baker for his help and the use of his apparatus.

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THE EFFECT OF TETRA-SODIUM 2-METHYL-1 : 4-NAPHTHOHYDROQUINIONE DIPHOSPHATE (SYNKAYVITE) ON A VARIETY OF MALIGNANT TUMOURS IN EXPERIMENTAL ANIMALS.

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MITCHELL (1948) recently reported a clinical study on the use of parenterally administered tetra-sodium 2-methyl-1 : 4-naphthohydroquinone diphosphate (synkayvite) as an adjuvant to radiotherapy in the management of inoperable and far advanced malignant disease. In a small series he noted prolongation of the mean survival time of patients with inoperable carcinoma of the bronchus treated by the combination of chemical agent and irradiation when compared with that of patients who received X-ray therapy alone, which he found to be statistically significant.

The rationale for Mitchell's (1948) selection of this drug for clinical trial in combination with radiotherapy depended upon his observation that the chemical compound produced mitotic inhibition of chick fibroblasts in tissue culture.

Although there have been many laboratory studies on synkayvite as a vitamin K substitute, no investigations have been published on the effect of this compound on experimental neoplasms. On the basis of the claims made by Mitchell, it was considered of interest to determine whether synkayvite alone had any demonstrable effect on a variety of malignant tumours in experimental animals in order to provide a basis for clinical trials with the agent. This report will present the results of such experiments on four transplantable mouse tumours (three adenocarcinomas of the breast and an undifferentiated sarcoma), and the Brown-Pearce carcinoma in rabbits (undifferentiated squamous cell carcinoma).

MATERIALS AND METHODS.

Preliminary chronic toxicity studies in mice were performed in the usual way to determine the maximal tolerated dose on which the animals could continue to maintain their body weight. Similar determinations were made in rabbits.

All of the mouse tumours were transplanted subcutaneously in the axillary region by the trocar method. The intraperitoneal administration of synkayvite was initiated 24 hours after transplantation, and continued daily for the duration of the experiment. Synkayvite was generously supplied by the Medical Division of Hoffman-La Roche, Inc., Nutley, N.J. The growth of the tumours in control and treated animals was followed by serial mensuration of the outside diameters, but the conclusions reached in this study rest on the comparison of the wet weight of the tumours in the control and treated series determined at the

termination of the experiment. All tumours were prepared for histological examination.

The effect of intraperitoneally injected synkayvite on the Brown-Pearce carcinoma was determined on transplants of the tumour in the anterior chamber of the eye in rabbits. This is a very convenient method for studying the action of potential chemotherapeutic agents on this tumour, inasmuch as the fragments grow uniformly, and completely fill the anterior chamber of the eye in 9–12 days. The growth of the tumour can be readily charted at daily intervals, and at the termination of the experiment the tumour, together with the infiltrated iris, is removed and weighed so that an objective, quantitative comparison between control and treated animals can be made. Results of the action of another compound on the Brown-Pearce carcinoma studied in this way have been reported (Gellhorn, Engleman, Shapiro, Graff and Gillespie, 1950).

RESULTS.

The toxicity experiments on mice and rabbits were not exhaustively pursued because, as will be seen, the results on the neoplasms failed to indicate any significant effect following the administration of synkayvite.

The results of experimental chemotherapy with synkayvite on a variety of neoplasms are summarized in Table I. From the information included it is possible to estimate with accuracy the actual protocols for the individual experiments, even though the pertinent results are here summarized as averages; the standard deviation has been calculated in each instance in order to describe the range and the distribution of the individual tumour weights about the mean tumour weight. The standard deviation of the mean which is necessary for the determination of statistical significance between two means is derived by dividing the standard deviation by the square root of the number of observations.

In several of the experiments 5-amino-7-hydroxy-1-H-v-triazolo [d] pyrimidine (8-azaguanine) was also administered to tumour-bearing animals for purposes of comparison with synkayvite. A complete report of our results with this chemical compound has been published (Gellhorn, Engleman, Shapiro, Graff and Gillespie, 1950). As can be seen from the tabular material, no significant difference was observed between the tumour weights of control and synkayvite treated mice. Statistical analysis of the results of synkayvite treatment in rabbits with the Brown-Pearce tumour was not made because of the small number of observations; however, it is immediately apparent that no effect was demonstrable.

Careful study of microscopic sections did not show any morphological changes which distinguished the tumours from control and treated animals.

DISCUSSION.

From the results presented in the foregoing section it must be concluded that synkayvite completely fails to inhibit the growth of three different types of mouse breast carcinomas, an undifferentiated squamous cell carcinoma in rabbits, and an undifferentiated sarcoma in mice. In the absence of any demonstrable carcinolytic or carcinostatic activity against experimental neoplasms the application of synkayvite alone in the therapy of human cancer would appear to be questionable. Although it is recognized that the parallelism between human

TABLE I.—*Effect of Synkayvite on a Variety of Malignant Tumours in Experimental Animals.*

Exp. No.	Neoplasm.	Type of tumour.	Group.	Number of animals.	Chemical compound.*	Dosage mg./kg./day.	Duration of therapy (days).	Mean tumour weight (mg.).	Standard deviation (mg.).	Standard deviation of mean (mg.).	Average body-weight	
											Beginning (g.).	End (g.).
1	755	Adenocarcinoma of breast in C57 mice	Control	10	—	—	—	638	258	82	18·1	19·6
			Treated	8	TP	50	18	13	9	3	16·0	16·4
				27	SYN	125	18	455	239	46	16·9	18·2
2	755	Ditto	Control	9	—	—	—	620	398	133	21·3	21·6
			Treated	9	TP	50	19	17	8	3	17·3	16·7
			„	29	SYN	125	19	515	241	45	19·1	20·1
3	RC	Adenocarcinoma of breast in dba mice	Control	10	—	—	—	652	444	140	24·7	25·5
			Treated	10	SYN	125	13	385	208	66	22·3	22·8
4	RC	Ditto	Control	10	—	—	—	405	177	56	13·5	15·5
			Treated	10	SYN	125	12	564	133	42	14·6	17·1
5	Eo771	Adenocarcinoma of breast in C57 mice	Control	10	—	—	—	535	300	95	10·0	19·7
			Treated	10	TP	50	11	116	56	18	20·2	19·8
			„	29	SYN	125	11	566	270	50	20·0	21·0
6	Sa. 180	Undifferentiated sarcoma in Long-acre mice	Control	10	—	—	—	299	150	48	24·5	24·0
			Treated	28	SYN	125	10	268	133	25	25·3	24·0
7	Brown-Pearce	Undifferentiated squamous cell carcinoma in rabbits	Control	9	—	—	—	792				
			Treated	10	TP	25	7	158				
			„	4	SYN	50	7	760				

* TP = 5-amino-7-hydroxy-14-v-triazolo [d] pyrimidine. SYN = Synkayvite.

neoplastic disease and malignant tumours in experimental animals is an unsettled issue, it is important to note that, thus far, all of the chemotherapeutic agents which have a demonstrable palliative effect against neoplasms in man have also been shown to inhibit comparable tumours in laboratory animals.

It is also appreciated that the observations which we have recorded do not duplicate in the laboratory the clinical experiments of Mitchell (1948), inasmuch as he studied the combination of radiotherapy and the drug whereas we investigated the drug alone. Evaluation of combination therapy, however, utilizing radiant energy plus a chemical compound, is not feasible in studies on experimental neoplasms due to the ease with which X rays alone can destroy subcutaneous tumours. Therefore, in spite of negative experimental evidence of the type presented in this report, the possibility remains that synkayvite in combination with radiotherapy may produce a greater carcinolytic effect than X-ray therapy alone. On examination of the available clinical evidence, it is difficult to assess the contribution offered by the therapeutic regimen of synkayvite and radiotherapy.

In Mitchell's (1948) report 73 patients with various types of advanced malignant tumours received intramuscular injections of the drug in combination with X-ray. Of this group, at least 23 patients were considered to have had an unexpectedly good palliative response to irradiation. However, since these cases included cancers of the mouth, lip, larynx, post-cricoid region, breast, tongue, nasal fossa and skin together with one osteosarcoma of the ilium, and since no criteria were recorded on which the designation of " unexpectedly good palliative response " was based, it is impossible to evaluate the results objectively. In another group of 10 patients representing a variety of advanced malignant diseases, the compound was administered intravenously and was the only form of therapy. Four of the individuals in this group experienced subjective relief, but no indication that the fundamental course of the disease had been altered by the therapy was indicated.

The main evidence for Dr. Mitchell's conclusion, that " tetra-sodium 2-methyl-1 : 4-naphthohydroquinone diphosphate in large doses produces a small but useful improvement in the palliative results of X-ray therapy in some cases of advanced cancer," rests on the results of combined treatment of 9 cases of bronchogenic carcinoma compared to 13 other patients with this disease treated by X-ray alone. In this group the mean survival time after the institution of radiotherapy or chemo- plus radio-therapy was 4·23 months for the controls and 7·56 months for the experimentals. The difference in the survival time was found to be significantly different by statistical methods. Before interpreting the mathematical analysis as an indication of biological significance, it must be recognized that the validity of the statistical technique rests on certain factors which, in this particular problem, include the assumptions that the probable duration of the disease was similar in the control and experimental series, that the histological types of neoplasm (which are known to influence the rapidity of progression of the tumours) were appropriately balanced between the two groups, and that other organic diseases which could influence the survival time played no part in the results. It is, of course, possible that these several variables were adequately controlled; however, in the absence of documentation in the report, the statistical evaluation of the clinical results can not be accepted without qualification.

SUMMARY AND CONCLUSIONS.

The effect of the vitamin K substitute tetra-sodium 2-methyl-1 : 4-naphthohydroquinone diphosphate (synkayvite) on the growth of five malignant, transplantable tumours in experimental animals was determined. No inhibitory action on three different types of adenocarcinoma of the breast, one undifferentiated squamous-cell carcinoma and one undifferentiated sarcoma could be demonstrated. There were no morphological changes in the tumours of animals treated with synkayvite which distinguished them from tumour sections of untreated control animals.

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ON THE SUSCEPTIBILITY OF THE THYMUS, LUNG, SUBCUTANEOUS AND MAMMARY TISSUES IN STRAIN STREET MICE TO DIRECT APPLICATION OF SMALL DOSES OF FOUR DIFFERENT CARCINOGENIC HYDROCARBONS.

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COMPARATIVE investigations on the potency of various carcinogenic hydrocarbons applied to tissues other than the skin and subcutaneous tissue are few. Such as have been performed involve, in most cases, the application of comparatively large doses of hydrocarbons with the intention of inducing as high an incidence of tumours as possible. Investigations on the relative carcinogenic potency of different hydrocarbons applied in submaximal doses to other tissues than the skin and subcutaneous tissue are not to hand.

In previous papers (Engelbreth-Holm and Rask-Nielsen, 1947 ; Rask-Nielsen, 1948) investigations carried out on a rather big scale have been reported, showing that different tissues in the Street strain of mice were not equally susceptible to the slight carcinogenic action of 0·02 mg. of 9:10:dimethyl-1:2-benzanthracene ; specific tumours of the thymus and lung, which are prone to spontaneous tumour development in this strain, were induced by this treatment, whereas the mammary and subcutaneous tissues which are also prone to spontaneous tumour development did not respond. The testis and kidney, in which spontaneous tumours have never been observed, and the spleen, lymph nodes and bone marrow, in which tumours are not presumed to develop spontaneously, also proved insusceptible to this slight carcinogenic influence, and did not respond to a very powerful direct carcinogenic influence. On the basis of these findings it was considered advisable to investigate the susceptibility of Street mice to the direct application of small doses of other carcinogenic hydrocarbons, particularly in the tissues prone to spontaneous development of tumours. This paper gives a report on such investigations on the susceptibility of the thymus, lung, mammary and subcutaneous tissues in Street mice to direct application of 0·02 mg. of the hydrocarbons most commonly dealt with, viz. 3:4-benzpyrene, 1:2:5:6-dibenzanthracene and 20-methylcholanthrene. Previous experiments applying 0·02 mg. 9:10-dimethyl-1:2-benzanthracene (Rask-Nielsen, 1948) are included for comparison.

MATERIAL AND METHODS.

Benzpyrene, dibenzanthracene, or methylcholanthrene was injected in a dose of 0·02 mg. dissolved in 0·01 c.c. paraffin of the same batch as that used for analogous experiments performed with 9:10-dimethyl-1:2-benzanthracene (Engelbreth-Holm and Rask-Nielsen, 1947 ; Rask-Nielsen, 1948). The technique of application was the same as that used in earlier experiments. When applied to

the thymus the needle was inserted at the upper border of the manubrium sterni and plunged downwards about 2 to 3 mm. immediately behind the sternum, where the hydrocarbon was deposited. When the hydrocarbon was applied to the lung, the needle was inserted through the abdominal wall immediately below the right costal border, through the diaphragm and through about two-thirds of the lung, thus making the path of the needle as long as possible. In the third experimental group, the hydrocarbon was deposited subcutaneously below the second lowest nipple, thus making it possible to observe the susceptibility of the mammary as well as of the subcutaneous tissue.

Litters of Street mice, 4 to 7 weeks old, were used exclusively. In the experiments comprising injections into the thymus and lung the mice were apportioned into four batches. One batch was left untreated to serve as a control group, and each of the remaining three batches was injected with one of the three hydrocarbons. As far as possible the litters were apportioned fairly equally to the four batches, each including about equal numbers of male and female mice. For the experiments on subcutaneous injection into the mammary region, litters of female mice were used; they were apportioned into two lots containing equal numbers, including controls and experimental animals, respectively. The experiments on subcutaneous injection of the three hydrocarbons were thus carried out in succession, whereas the experiments comprising injections into the thymus and lung were carried out simultaneously and on mice from the same litters. The female mice were not allowed to breed.

The animals were fed on whole meat and rolled oats with the addition of cod-liver oil and yeast once a week; the supply of drinking water was unrestricted. All the mice were autopsied, and all tumours, together with liver, spleen, kidney, lung and enlarged lymph nodes from animals showing leukaemic lesions, including those showing isolated thymic hyperplasias, were examined microscopically.

RESULTS.

The incidence of spontaneous tumours in the controls is recorded in Table I; leukaemia was found in 1·6 per cent, mammary carcinomas in non-breeding

TABLE I.—*Tumours Observed in the Controls.*

	Incidence.	Age in months.
Leukaemia	11/681, 1·6%	3, 5, 9, 15, 17, 19, 19, 19, 21, 23, 23
Mammary carcinoma in non-breeding females	1/176, 0·6%	10
Pulmonary adenoma	1/41, 2·4%	21
Subcutaneous tumour	4/172, 2·3%	12, 13, 16, 32

females in 0·6 per cent, pulmonary adenomas in 2·7 per cent and subcutaneous tumours in 2·3 per cent of the animals. These findings are in a fair accordance with previous findings in Street mice (Rask-Nielsen, 1948; Lefèvre, 1945).

The number and survival time of the animals included in the experiments are recorded in Table II and the incidence of local and remote tumours is recorded in Table III.

Thymic tumours.

As appears from Table III, injections of 0·02 mg. of the four hydrocarbons—benzpyrene, dibenzanthracene, methylcholanthrene and 9:10-dimethyl-1:2-ben-

zanthracene—induced specific tumours, lymphosarcomatous hyperplasia of the thymic gland in 1·7 per cent, 6·7 per cent, 10·4 per cent and 13 per cent, the effective total being the number of mice whose survival time was at least that of the youngest tumour-bearing animal within all four groups, namely, three months. In addition a thymic spindle-cell sarcoma was found in one animal injected with methylcholanthrene.

Injections of three of the hydrocarbons into the lung induced thymic tumours in 2, 2 and 4 per cent following the injection of benzpyrene, dibenzanthracene and 9:10-dimethyl-1:2-benzanthracene respectively. No thymic tumours developed after injection of methylcholanthrene. Because of the close relative position of the thymus and lung this tumour development is probably to be considered a local one.

TABLE II.—*Number and Survival Time of Experimental Mice.*

Site of injection.	0·02 mg. of—	Months. 3.	6.	9.	12.	15.	18.	21.	24.	27.	30.
Thymus.	Benzpyrene	58	36	26	19	11	8	5	3	0	—
	Dibenzanthracene	75	46	21	15	8	5	2	1	0	—
	Methylcholanthrene	77	54	26	17	10	5	1	1	0	—
	9:10-Dimethyl-1:2-benzanthracene	68	31	25	15	14	11	6	4	3	0
Lung.	Benzpyrene	66	43	22	18	12	5	2	1	0	—
	Dibenzanthracene	80	44	20	14	12	8	2	1	0	—
	Methylcholanthrene	73	54	24	17	7	5	3	2	0	—
	9:10-Dimethyl-1:2-benzanthracene	108	64	48	34	28	16	10	6	0	—
Mammary region.	Benzpyrene	59	21	18	17	11	11	8	6	3	0
	Dibenzanthracene	46	28	20	20	19	17	14	9	3	0
	Methylcholanthrene	48	37	20	11	6	6	2	1	0	—
	9:10-Dimethyl-1:2-benzanthracene	57	22	11	6	4	0	—	—	—	—

The survival time of the tumour-bearing animals is also recorded in Table III. The average latent period, the interval between injection and the death of the animal, following injections into the thymus of benzpyrene, dibenzanthracene, methylcholanthrene, and 9:10-dimethyl-1:2-benzanthracene was 15, 14, 16 and 21 weeks, respectively; the minimum latent period of the three latter hydrocarbons was 11, 14 and 10 weeks, respectively. The latent period following injection into the lung seems to be slightly longer than the latent period following injection into the thymus but the small number of animals involved does not allow of any further deductions to be made.

It thus appears that the thymus proved susceptible to all four hydrocarbons in the very small dose applied, and moreover that the carcinogenic effect of the hydrocarbons on the thymus was in descending order, 9:10-dimethyl-1:2-benzanthracene, methylcholanthrene, dibenzanthracene, benzpyrene, with 9:10-dimethyl-1:2-benzanthracene and methylcholanthrene almost equal.

A comparison with the controls in which 9 cases of generalized leukaemia without thymic tumours, 1 case of isolated thymic tumour (in a 3-months-old mouse), and 1 case of generalized leukaemia associated with thymic tumour (in a 9-months-old mouse) were observed among 681 animals, shows that the number of induced thymic tumours by far exceeds that of spontaneous tumours.

Apart from the 28 isolated thymic hyperplasias, generalized leukaemias were observed in 11 experimental animals following injections into the thymus and

lung (Table III); in two of these animals, aged 11 and 14 months, severe generalized leukaemic lesions associated with thymic tumours developed. These two cases, and probably the other generalized leukaemias as well, may have developed through secondary infiltration of malignant leukaemic cells originating from the thymus and there induced by the carcinogenic effect. However, the

TABLE III.—*Tumours Observed in Experimental Mice.*

Site of injection.	0·02 mg. of—	Local tumours.					
		Thymic lymphosarcoma.		Spindle-cell sarcoma at site of injection.		Pulmonary adenoma.	
		Incidence.	Age in months.	Incidence.	Age in months.	Incidence.	Age in months.
Thymus.	Benzpyrene	1/58, 1·7%	5	—	—	0/36, 0%	—
	Dibenzanthracene	5/75, 6·7%	4, 5, 5, 5, 7	—	—	1/46, 2%	23
	Methylcholanthrene	8/77, 10·4%	5, 5, 5, 5, 5, 6, 6, 6	1/77, 1·3%	8	2/54, 3·7%	6, 19
	9:10-Dimethyl-1:2-benzanthracene	9/68, 13%	3, 3, 3, 4, 4, 5, 7, 8, 13	—	—	2/31, 6·5%	19, 29
Lung.	Benzpyrene	1/46, 2%	6	—	—	0/18, 0%	—
	Dibenzanthracene	1/52, 2%	5	—	—	4/16, 25%	12, 17, 20, 20
	Methylcholanthrene	0/59, 0%	—	—	—	1/17, 6%	20
	9:10-dimethyl-1:2-benzanthracene	3/73, 4%	5, 7, 12	—	—	6/37, 16%	11, 15, 21, 25, 25, 26
Mammary region.	Benzpyrene	—	—	0/26	—	—	—
	Dibenzanthracene	—	—	0/29	—	—	—
	Methylcholanthrene	—	—	4/38, 10%	5, 5, 8, 12	—	—
	9:10-Dimethyl-1:2-benzanthracene	—	—	0/44	—	—	—

Site of injection.	0·02 mg. of—	Non-local tumours.							
		Pulmonary adenoma.		Generalized leukaemia.		Mammary carcinoma.		Various tumours.	
		Incidence.	Age in months.	Number of tumours.	Age in months.	Number of tumours.	Age in months.	Number of tumours.	Age in months.
Thymus.	Benzpyrene	—	—	1	14	—	—	—	—
	Dibenzanthracene	—	—	1	11	—	—	—	—
	Methylcholanthrene	—	—	1	12	1	17	1	17 (hepatoma)
	9:10-Dimethyl-1:2-benzanthracene	—	—	—	—	1	16	—	—
Lung.	Benzpyrene	—	—	4	7, 14, 15, 15	—	—	1	7 (perirenal plasmacytoma)
	Dibenzanthracene	—	—	2	16, 17	2	16, 16	—	—
	Methylcholanthrene	—	—	2	8, 20	—	—	—	—
	9:10-Dimethyl-1:2-benzanthracene	—	—	—	—	2	16, 16	1	17 (inguinal spindle-cell sarcoma)
Mammary region.	Benzpyrene	—	—	1	21	5	17, 18, 19, 23, 23	—	—
	Dibenzanthracene	2	19, 24	2	22, 24	3	22, 22, 24	—	—
	Methylcholanthrene	1	11	2	18, 25	—	—	—	—
	9:10-Dimethyl-1:2-benzanthracene	—	—	—	—	1	15	2	13 (inguinal spindle-cell sarcoma) 15 (mesenteric lymphosarcoma)

long survival time and the comparatively small number of animals involved rather indicate that these cases are spontaneous leukaemias. In support of this view is also the fact that the induced isolated thymic hyperplasias showed only a local sarcomatous growth without development of macroscopical leukaemic lesions, and with only slightly pronounced microscopical lesions in other organs, except for leukaemic infiltrations in the lung. Leukaemic infiltrations in the liver, for instance, were seen only in 2 out of 28 experimental animals suffering from isolated thymic tumours.

All the thymic tumours and generalized leukaemias were of the stem-cell variety with the exception of two cases of generalized plasma-cell leukaemias.

Pulmonary adenomas.

Table III indicates that in addition to the above-mentioned thymic tumours in mice injected in the lung, pulmonary adenomas of the right lung following the injection of dibenzanthracene, methylcholanthrene and 9:10-dimethyl-1:2-benzanthracene were observed in 25, 6 and 16 per cent respectively, the effective total being the number of experimental mice attaining the same age as the youngest adenoma-bearing animal in all four groups, namely, 11 months. Benzpyrene caused no development of pulmonary adenomas.

Development of pulmonary adenomas was also seen following injection into the thymus in 2, 3·7 and 6·5 per cent respectively with dibenzanthracene, methylcholanthrene and 9:10-dimethyl-1:2-benzanthracene. Benzpyrene injection caused no development of pulmonary adenomas.

A comparison with the development of pulmonary adenomas in the controls (Table I) as well as in the controls of previous experiments (Rask-Nielsen, 1948) where the incidence of pulmonary adenomas was slightly higher, at 2·4 per cent in animals aged 12–24 months, shows that the carcinogens did not shorten the latent period of pulmonary adenomas ; the incidence of these tumours increased, however, when 9:10-dimethyl-1:2-benzanthracene, and in particular when dibenzanthracene injection into the lung was applied. Evidently, this did not happen after application to the lung of the two other hydrocarbons, or after application to the thymus of any of the hydrocarbons. There was, however, one case of pulmonary adenoma in a 20-months-old animal following methylcholanthrene injection which may have developed spontaneously. The experiments thus show that the pulmonary tissue was susceptible to direct application of 0·02 mg. of two out of the four hydrocarbons concerned, dibenzanthracene and 9:10-dimethyl-1:2-benzanthracene ; the carcinogenic potency of the former was greater than that of the latter.

Among the 16 pulmonary tumours observed, 12 were typical adenomas or beginning carcinomas and 4 were definite adeno-carcinomas. Of the 11 cases of pulmonary tumours, 8 developed in the right lung, the site of injection ; the remaining three cases showed adenomas in both lungs. Moreover, typical pulmonary adenomas of the left lung were observed in 2 animals aged 12 and 23 months, and these cases were probably spontaneous. All adenomas were visible macroscopically, but were microscopically verified. No consideration has thus been paid to microscopical adenomas, if any.

A few mammary carcinomas and other tumours have also been observed in the experiments applying injections into the thymus or lung (Table III) ; probably they are to be considered spontaneous cases.

Subcutaneous spindle-cell sarcomas.

As appears from Table III, local spindle-cell carcomas developed in 4 out of 38 animals (10 per cent) following subcutaneous injection of methylcholanthrene below the second lowest mammary nipple, whereas no sarcomas occurred after injections of the other three hydrocarbons. The experiment indicates that the subcutaneous tissue was susceptible to the action of methylcholanthrene but not to the action of the other three hydrocarbons in the doses applied. The carcinogenicity of methylcholanthrene should therefore be considered superior to that of the three other hydrocarbons as regards subcutaneous tissue.

Mammary carcinomas.

Table III indicates that five mammary carcinomas were found in the benzpyrene group, three in the dibenzanthracene group, and one in the animals treated with 9:10-dimethyl-1:2-benzanthracene, the survival time of the animals being from 15–24 months. However, none of the 9 tumours developed exactly at the site of injection, and only in two cases so close to this site as to indicate any local tumour development. It is rather to be assumed that all 9 tumours developed spontaneously, an assumption which is also supported by the rather long survival time of the animals. It should be observed that the incidence of mammary carcinomas, 9 tumours, or 14 per cent, among 64 females, supersedes that of the controls. Probably it may be a mere coincidence, because the incidence of mammary carcinomas in the controls of these experiments has been very low, even lower than that of the controls in previous experiments (Rask-Nielsen, 1948 ; Lefèvre, 1945) where the incidence in non-breeding females was 2·3 per cent and 7 per cent respectively, and also lower than that of controls in various other experiments carried out simultaneously with those here dealt with and in which the incidence was 9·4 per cent. Considering the fluctuation of the mammary carcinoma incidence it seems less probable that the mammary carcinomas obobserved in the experimental animals might indicate any acceleration of this tumour development, but rather that they have developed spontaneously. Evidently, the mammary tissue has not proved susceptible to the action exerted by any of the four hydrocarbons in the doses applied.

Finally, three cases of pulmonary adenomas, five cases of generalized leukaemias and one case of mesenteric lymphosarcoma were observed in this experiment. There is no doubt that they should be regarded as spontaneous tumours, and presumably the same applies to a spindle-cell sarcoma in the right inguinal region, even if the possibility of it being a local tumour cannot be excluded.

It is shown by the experiments not only that the four tissues examined—all of which are prone to spontaneous development of tumours in Street mice—exhibited obviously different susceptibility to a weak carcinogenic action, but also that the relative carcinogenicity of the four hydrocarbons applied has been different for the various tissues examined. The thymus proved susceptible to all four hydrocarbons with decreasing activity in the following order : 9:10-dimethyl-1:2-benzanthracene, methylcholanthrene, dibenzanthracene, benzpyrene ; pulmonary tissue only to dibenzanthracene and, to a slighter degree, to 9:10-dimethyl-1:2-benzanthracene ; subcutaneous tissue was susceptible only to methylcholanthrene, and mammary tissue not to any of the hydrocarbons concerned.

It should be noted that apart from one thymic spindle-cell sarcoma, all the

local tumours were specific tumours of the tissue concerned. This is in contrast to the tumour development induced by application to the lung of large doses of benzpyrene, dibenzanthracene, and methylcholanthrene, which for the greater part induced spindle-cell sarcomas (Rask-Nielsen, 1950*a*).

DISCUSSION.

Elucidation of the susceptibility of certain tissues to various hydrocarbons under identical experimental conditions has been the object of the investigations reported in this paper. They have supported the previous observation (Rask-Nielsen, 1948), that the susceptibility to a small dose of a carcinogenic hydrocarbon is different in various tissues prone to spontaneous tumour development in Street mice. Moreover, the experiments here dealt with have proved that the effect of the four hydrocarbons was the same since the thymus, the tissue considered the most susceptible, was influenced by all four hydrocarbons, even in the small doses applied, and the mammary tissue, which, at any rate under the experimental conditions in question, was considered the least susceptible, was not influenced by any of the four hydrocarbons, and even in large doses of 0·5 mg. the four hydrocarbons have not induced any specific tumours in the mammary tissue (Rask-Nielsen, 1950*b*). As to the susceptibility of the subcutaneous tissue, which was found susceptible to methylcholanthrene only, it must be fairly obvious to presume that the dose of the other three hydrocarbons has been below the level of the susceptibility of the subcutaneous tissue. To assume any insusceptibility of that tissue to those three hydrocarbons is out of the question, since large doses of these hydrocarbons have induced local sarcomas (Rask-Nielsen, 1950*b*). The same may also apply to pulmonary tissue, which in the present experiments has proved susceptible to dibenzanthracene and 9:10-dimethyl-1:2-benzanthracene, and the dose of the two other hydrocarbons has probably been below the level of susceptibility of the tissue. Experiments involving injections of large doses of these hydrocarbons to the lung support this contention (Rask-Nielsen, 1950*a*). The experiments thus indicate that three of the tissues examined have been susceptible to the action of all four hydrocarbons and that the mammary tissue is not susceptible to any of them.

Moreover, it will be noted that the relative potency of the four hydrocarbons, estimated on the basis of the tumour incidence, the latent period being of almost equal length, has been different for the various tissues examined. As to the thymus the carcinogenic potency was in descending order 9:10-dimethyl-1:2-benzanthracene, methylcholanthrene, dibenzanthracene, benzpyrene; pulmonary tissue proved susceptible to dibenzanthracene and, to a slighter degree, to 9:10-dimethyl-1:2-benzanthracene, and subcutaneous tissue only to methylcholanthrene. Such differences in relative carcinogenicity for the various tissues have not previously been reported by others. Andervont and Shimkin (1940) observed that the relative descending order of potency for three of the hydrocarbons for both skin and subcutaneous tumours in A mice was methylcholanthrene, benzpyrene, dibenzanthracene, but for lung tumours it was dibenzanthracene, methylcholanthrene, benzpyrene. The particular susceptibility to dibenzanthracene of the pulmonary tissue in Street mice reported in this paper is in accordance with their findings.

9:10-Dimethyl-1:2-benzanthracene is the most potent of all carcinogenic hydrocarbons when applied cutaneously (Bachmann, Kennaway and Kennaway,

1938; Iball, 1939), as opposed to subcutaneous injection, which induces only a few local tumours (Shear, 1938; Engelbreth-Holm and Lefèvre, 1941; Rask-Nielsen, 1948), by far less than are caused by administration of methylcholanthrene, the carcinogenicity of which is superior to that of 9:10-dimethyl-1:2-benzanthracene when applied to subcutaneous tissue, but inferior when applied to the epidermis. As to the potency of the other hydrocarbons for subcutaneous tissue it should be observed that some investigators (Bryan and Shimkin, 1943; Shimkin and Wyman, 1947) found that the carcinogenic effect of large doses applied to C_3H mice was in descending order, methylcholanthrene, benzpyrene, dibenzanthracene, the order in which the carcinogenicity of these hydrocarbons is usually referred to in the literature, but when applied in very small doses dibenzanthracene seems to exhibit greater carcinogenic potency than do methylcholanthrene and benzpyrene. In accordance with the above Shear and Lorenz (1939) using strain A mice, and Dobrovolskaïa-Zavadskaïa (1938), using mice of unnamed strain, succeeded in inducing tumours by the application of very small doses of dibenzanthracene. Nothing similar has been found in the experiments on Street mice here dealt with, only methylcholanthrene causing development of tumours in the dose applied.

Since positive results of inducing thymic tumours by means of direct application of carcinogenic hydrocarbons are not available from other laboratories, comparisons cannot be instituted. It should therefore only be emphasized that the particularly high susceptibility of the thymus to 9:10-dimethyl-1:2-benzanthracene may be a concurrent cause to the very extensive development of leukaemia in Street mice induced by cutaneous (Lefèvre, 1945) as well as by subcutaneous application (Rask-Nielsen, 1949, 1950*b*), since even the absorption of very small quantities of hydrocarbon is presumed to induce thymic lymphosarcoma and even generalized leukaemia, provided that leukaemic lesions in other organs are developed through secondary infiltration of cells from the thymus. Previous investigations (McEndy, Boon and Furth, 1944; Kaplan, 1947, 1948; Rask-Nielsen, 1948) are in support of this contention.

It has already been mentioned that the mammary tissue was shown to be not susceptible to the local action of 0·02 mg. in the present investigations, or 0·5 mg. (Rask-Nielsen, 1950*b*) of the four hydrocarbons concerned. Analogous negative results were observed by others, using mice from various strains (Esmarch, 1940; Strong and Smith, 1939). Experiments including the NHO strain of mice, however, exhibit positive results (Strong and Smith, 1939; Strong and Williams, 1941; Strong, 1945). From these experiments it is rather difficult to form an estimate of the susceptibility of the mammary tissue to carcinogenic hydrocarbons, since susceptibility is dependent on simultaneous hormonal stimulation as well as on the presence of the milk agent. Since the present experiments included only non-breeding mice the hormonal stimulation was at least not maximal.

Street mice have been used in all the experiments recorded here and the deductions made apply only to mice of that strain. The susceptibility to carcinogenic action of various tissues being genetically determined (Lefèvre, 1945) it is necessary, in order to elucidate the susceptibility to a direct carcinogenic action of a particular tissue, to examine animals from different strains under identical experimental conditions. Such investigations, for the time being with application of 9:10-dimethyl-1:2-benzanthracene to the thymus, lung and subcutaneous

tissue below a mammary nipple in dlb mice and C_3H mice, are now in progress and will be published later.

SUMMARY.

Investigations into the susceptibility of the thymus, lung, subcutaneous and mammary tissue of Street mice to the direct application of the small dose of 0·02 mg. of benzpyrene, dibenzanthracene, methylcholanthrene, and 9:10-dimethyl-1:2-benzanthracene are reported. The thymus was susceptible to all four hydrocarbons and pulmonary tissue was susceptible to dibenzanthracene, 9:10-dimethyl-1:2-benzanthracene and methylcholanthrene. Subcutaneous tissue was susceptible only to methylcholanthrene and mammary tissue was susceptible to none of the hydrocarbons.

With the exception of one thymic spindle-cell sarcoma, only specific tumours were induced. It is emphasized that the susceptibility to a weak carcinogenic action differed for the various tissues examined, and the order of the relative carcinogenic potency of the four hydrocarbons varied for the tissues which proved susceptible.

This means that when estimating the carcinogenic potency of the hydrocarbons regard must be paid not only to the well known fact that a particular tissue may respond differently in mice of different strains and that various tissues in mice from one particular strain may respond differently to the application of a particular hydrocarbon, as shown by us earlier, but also to the fact that these tissues in one particular strain may respond differently to different hydrocarbons administered in doses of identical weight.

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TYPES OF TUMOURS IN THE LUNGS OF STRAIN STREET MICE FOLLOWING DIRECT APPLICATION OF LARGE DOSES OF FOUR DIFFERENT CARCINOGENIC HYDROCARBONS.

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EXPERIMENTS involving direct application of the large dose of 0·5 mg. of 9:10-dimethyl-1:2-benzanthracene into the lungs of mice of strain Street formed a part of a comprehensive study (Rask-Nielsen, 1948) carried out in an endeavour to confirm or repudiate the hypothesis that tumours can be induced only in tissues that produce tumours spontaneously, whereas tissues that do not produce tumours spontaneously are unable to develop tumours even following very powerful carcinogenic action. The experiments showed that direct application of 0·5 mg. of 9:10-dimethyl-1:2-benzanthracene to mice of the strain used induced specific tumours of the spontaneously tumour-producing tissues, thymus, lung and subcutaneous tissue, but failed to affect the mammary tissue which also develops spontaneous tumours. With the exception of one testicular tumour, specific tumours were not induced in the spleen and lymph nodes, tissues which were presumed to develop no spontaneous tumours, or in kidney and testes, tissues known to produce no spontaneous tumours. Moreover, non-specific tumours, in the form of spindle-cell sarcomas, were observed only in experiments where they could have originated in the subcutaneous tissue and not in those where they would have to be derived from the interstitial connective tissue of the organ. Thus, direct application of 9:10-dimethyl-1:2-benzanthracene, 0·5 mg., to the lung, kidney and spleen did not induce spindle-cell sarcomas in these organs.

Attempts were then made to ascertain whether and to what extent other carcinogenic hydrocarbons, under the same experimental conditions, would induce specific tumours in some of the organs studied, including the lung, and whether such hydrocarbons, unlike 9:10-dimethyl-1:2-benzanthracene, would be able to induce non-specific spindle-cell sarcomas in the lung. To this end, 3:4-benzpyrene, 1:2:5:6 dibenzanthracene, or 20-methylcholanthrene, all in doses of 0·5 mg., were introduced directly into the lungs of Street mice.

Since application of 9:10-dimethyl-1:2-benzanthracene to the lung had previously induced thymic tumours (Rask-Nielsen, 1948), the present experiments were presumed to afford information also about the tumours which the three hydrocarbons would be capable of inducing in the thymus.

These experiments will be reported in the present paper, which also includes the previous experiments with 9:10-dimethyl-1:2-benzanthracene for comparison.

MATERIAL AND METHODS.

Benzpyrene, dibenzanthracene, or methylcholanthrene, 0·5 mg., was injected, suspended in 0·01 c.c. of the same mixture of hard and liquid paraffin as used in

the previous experiments (Rask-Nielsen, 1948). The technique was as follows: In order to make the path of the needle in the lung tissue as long as possible, the needle was inserted through the abdominal wall, immediately below the right costal border, and plunged through the diaphragm and longitudinally upwards through about two-thirds of the chest, before the suspension was injected.

The mice used were litter mates of strain Street, aged 5 to 7 weeks, divided into four lots. One lot was left untreated as controls, whereas the other three were injected with the respective hydrocarbons. As far as possible, the litters were divided equally in all four lots, an equal number of males and females in each lot. In the corresponding experiments carried out with 9:10-dimethyl-1:2-benzanthracene (Rask-Nielsen, 1948) litters of Street mice were divided into lots of equal number, one of which was set aside as a control group. The only spontaneous tumours observed among all the controls were two cases of pulmonary adenoma in mice aged 12 and 19 months, and six cases of mammary carcinoma in mice aged 13 to 22 months.

The mice were fed whole wheat and rolled oats with a weekly addition of cod liver oil and yeast. All dead mice were autopsied, and all grossly visible pulmonary and thymic growths were examined microscopically.

RESULTS.

Table I sets out the number and survival time of the experimental mice. It will be seen that only a few mice reached an advanced age. The high mortality

TABLE I.—*Number and Survival Time of the Experimental Mice.*

Injection of 0·5 mg.	Months.							
	3.	6.	9.	12.	15.	18.	21.	24.
Benzpyrene	85	71	29	20	17	7	2	0
Dibenzanthracene	79	45	8	6	1	0	—	—
Methylcholanthrene	77	34	6	2	1	0	—	—
9:10-Dimethyl-1:2-benzanthracene	83	23	6	3	0	—	—	—

was due partly to the induced growths, but partly also to the development of hydrothorax so severe that the lifetime of the mice was greatly reduced, even in the absence of tumours.

The growths observed are presented in Table II, which shows that thymic tumours were observed in addition to adenomas and spindle-cell sarcomas of the lung.

TABLE II.—*Incidence of Tumours.*

Injection of 0·5 mg.	Pulmonary tumours.						Thymic tumours.	
	Adenomas in the right lung.				Spindle-cell sarcomas.			
	Macroscopic.		Microscopic.					
	Number of tumours.	Age in months.	Number of tumours.	Age in months.	Incidence.	Age in months.	Incidence.	Age in months.
Benpyrene	2/20	15, 17	0/6	—	2/81, 2·5%	7–8	4/85, 4·7%	5–8
Dibenzanthacene	2/6	13, 16	4/21	5, 6, 6, 7	14/73 19%	4–12	7/79, 9%	4–7
Methylcholanthrene	0/2	—	2/29	6, 7	19/59, 32%	4–10	10/77, 13%	3–5
9:10-Dimethyl-1:2-benzanthracene	1/3	12	1/9	5	0/48	—	9/83, 11%	3–7

Pulmonary adenoma.

It is clear from Table II that macroscopic adenomas of the right lung, the site of the injection, were observed following injection of benzpyrene in only 2 out of 20 mice aged 15 and 17 months, following injection of dibenzanthracene in 2 out of 6 mice aged 13 and 16 months, and following injection of 9:10-dimethyl-1:2-benzanthracene in 1 out of 3 mice aged 12 months. The effective total is the number of mice living to be as old as the youngest tumour-bearing mouse, namely, 12 months.

In the sections prepared for the study of the spindle-cell sarcomas and thymic tumours, small adenomas were observed in a few mice. As shown in Table II, this phenomenon occurred following injection of dibenzanthracene in 4 out of 21 mice aged 5–7 months, following injection of methylcholanthrene in 2 out of 29 mice aged 6 and 7 months, and following injection of 9:10-dimethyl-1:2-benzanthracene in 1 out of 9 mice aged 5 months. These figures apply only to the adenomas, one, or at most two or three, in each mouse, observed in one section through the lungs. Nothing is known about microscopically visible adenomas in the remaining part of the lungs. Neither is it known whether a corresponding production of adenomas had taken place in the mice showing no grossly visible tumours, but it is considered probable. It is worth mentioning that untreated Street mice do not usually exhibit microscopic adenomas. Serial sections from 48 mice, ranging in age from four to twelve months (Rask-Nielsen, 1948), showed one adenoma, only microscopically visible, in a mouse aged 12 months. The present experiments, therefore, appear to indicate that 9:10-dimethyl-1:2-benzanthracene, methylcholanthrene, and dibenzanthracene, and presumably also benzpyrene, have induced pulmonary adenomas. The explanation why these growths were in most cases visible only upon microscopic examination is probably afforded by the short survival time of the mice (Table I). Although the experiments allow of but an estimate of the development of pulmonary adenoma, they appear to indicate that injection of dibenzanthracene has induced a more marked increase in the development of adenoma than did injection of benzpyrene, methylcholanthracene and 9:10-dimethyl-1:2-benzanthracene.

The macroscopic adenomas as well as those visible only upon microscopic examination were of the ordinary, typical sub-pleural variety.

In addition to the adenomas of the right lung, adenomas were also present in the left lung of a 7-month-old mouse injected with dibenzanthracene and in the two 12-month-old mice injected with 9:10-dimethyl-1:2-benzanthracene. A 5-month-old mouse, injected with 9:10-dimethyl-1:2-benzanthracene, exhibited adenomas only in the left lung. In this case the growths co-existed with a thymic tumour. It is debatable whether these adenomas of the left lung are to be interpreted as spontaneous growths or induced by the carcinogenic action.

Spindle-cell sarcomas.

In about half the instances, the growths were circumscribed, spherical swellings in the pulmonary tissue, and in the remaining cases the growth formed an adhesion between the right lung and the diaphragm, the chest wall or the inferior mediastinum. In these latter cases the tumours have probably developed along the entire path of the injection. All the growths were typical, ordinary spindle-cell

sarcomas of varying differentiation. In two mice the spindle-cell sarcomas were associated with lymphosarcomatous thymic tumours.

Table II shows that spindle-cell sarcomas were observed following injection of benzpyrene in 2 out of 81 (2·5 per cent), following injection of dibenzanthracene in 14 out of 73 (19 per cent), and following injection of methylcholanthrene in 19 out of 59 mice (32 per cent), the effective total of experimental mice being the number of mice living to be as old as the youngest tumour-bearing mouse of all 4 groups, namely four months. As mentioned above, spindle-cell sarcomas were not observed following injection of 9:10-dimethyl-1:2-benzanthracene.

The minimum, maximum and average latent period of the growths, the interval from injection until death, is set out in Table III. It will be seen that the

TABLE III.—*Minimum, Maximum and Average Latent Period (in weeks).*

Injection of 0·5 mg.	Spindle-cell sarcoma.			Thymic tumours.		
	Minimum.	Maximum.	Average.	Minimum.	Maximum.	Average.
Benzpyrene	25	28	26	14	25	20
Dibenzanthracene	13	45	23	12	24	16
Methylcholanthrene	7	32	16	7	13	10
9:10-Dimethyl 1:2-benzanthracene	—	—	—	9	27	16

average latent period of spindle-cell sarcoma following injection of benzpyrene, dibenzanthracene and methylcholanthrene was 26, 23 and 16 weeks respectively.

Thus, the experiments showed that the carcinogenicity of benzpyrene, dibenzanthracene, and methylcholanthrene, injected in doses of 0·5 mg., for the interstitial tissue of the lung increased in the order mentioned, and that the latent period of the growths decreased in the same order, whereas 9:10-dimethyl-1:2-benzanthracene—injected in the same dose—proved to be non-carcinogenic for the interstitial tissue of the lung.

Thymic tumours.

It will be seen from Table II that thymic tumours were observed following injection of benzpyrene into the lungs of 4 out of 85 (4·7 per cent), following injection of dibenzanthracene in 7 out of 79 (9 per cent), following injection of methylcholanthrene in 10 out of 77 (13 per cent) and following injection of 9:10-dimethyl-1:2-benzanthracene in 9 out of 83 mice (11 per cent). The effective total of experimental mice was calculated on the basis of the number of mice living to be as old as the youngest mouse exhibiting a thymic tumour within all four groups, namely three months. With the exception of one spindle-cell sarcoma of the thymus, observed in a mouse injected with methylcholanthrene, all the growths were typical lymphosarcomas, made up of stem cells and accompanied by a frequently highly pronounced perivascular infiltration, particularly in the central areas of the lungs. One mouse injected with 9:10-dimethyl-1:2-benzanthracene exhibited such violent infiltration without actual thymic tumour.

The latent period of thymic tumours is given in Table III, which shows that the average latent period following injection of benzpyrene, dibenzanthracene, methylcholanthrene, and 9:10-dimethyl-1:2-benzanthracene was 20, 16, 10 and 16 weeks respectively.

The experiments revealed that the carcinogenicity of the hydrocarbons used, in doses of 0·5 mg. introduced into the lung, for the thymus increased in the

order benzpyrene, dibenzanthracene, 9:10-dimethyl-1:2-benzanthracene, and methylcholanthrene and that the latent period decreased in the same order.

In addition to the local tumours, four cases of generalized leukaemia in mice aged 13 to 20 months, occurred among the 20 mice living to be 13 months of age in the benzpyrene group and one case of generalized leukaemia in a mouse aged 7 months among the 32 mice living to be at least 7 months of age in the dibenzanthracene group. Presumably these cases are to be interpreted as spontaneous phenomena, although it cannot be excluded that they may have been the result of secondary invasion of the various organs by malignant cells induced in the thymus by the carcinogenic action. The likelihood of such a pathogenesis of stem cell leukaemia in Street mice has been advanced previously (Rask-Nielsen, 1948). None of the six cases of generalized leukaemia was affected with thymic tumour.

On the whole, the experiments showed that the order of carcinogenicity of the four hydrocarbons, as estimated from the incidence of tumours induced, varied, when 0·5 mg. of the hydrocarbons was applied to the lungs of Street mice, for the different tissues subject to their action. In the case of the specific lung tissue, the carcinogenicity of dibenzanthracene proved higher than that of the other three hydrocarbons ; in the case of the interstitial tissue of the lungs, the carcinogenicity decreased in the order : methylcholanthrene, dibenzanthracene, benzpyrene to 9:10-dimethyl-1:2-benzanthracene which proved to be non-carcinogenic for this tissue ; in the case of thymic tissue, the order was methylcholanthrene, 9:10-dimethyl-1:2-benzanthracene, dibenzanthracene, benzpyrene. The duration of the latent period was reversely proportional to the carcinogenicity.

DISCUSSION.

Attempts at inducing tumours in the lung by direct application of carcinogenic hydrocarbons have been reported by a few earlier authors. According to these reports, direct injection of methylcholanthrene (Esmarch, 1940*a*, 1940*b*, 1942) produced almost exclusively spindle-cell sarcomas, whereas insertion of threads coated with dibenzanthracene into the lung (Andervont, 1937*a*), intratracheal administration of dibenzanthracene or methylcholanthrene (Shimkin, 1939) or tube feeding with dibenzanthracene (Magnus, 1939) produced practically only pulmonary adenomas. Moreover, intravenous injection of dibenzanthracene or methylcholanthrene, especially into strain A mice (Andervont and Lorenz, 1937 ; Andervont, 1939*a* ; Shimkin, 1940 ; Shimkin and Lorenz, 1942), and also into mice of other strains (Andervont, 1939*b*) has been found to produce pulmonary adenomas. This effect has also been reported following subcutaneous injection of carcinogenic hydrocarbons (Andervont, 1937*b*, 1938). It appears that not only the choice of hydrocarbon, but also the method of application and the genetic constitution of the strain used have influenced the type and extent of the tumour induction.

As regards the potency of methylcholanthrene to induce spindle-cell sarcomas following direct injection into the lung and the special potency of dibenzanthracene to induce pulmonary adenomas, the experiments reported in this paper accord with the experience of previous authors. On the other hand, the power of methylcholanthrene to induce pulmonary adenomas following intravenous injection was not observed following direct injection of the hydrocarbon into the lung.

The present experiments are in conformity with recently published studies on the susceptibility of lung tissue to the small dose of 0·02 mg. of the same four hydrocarbons (Rask-Nielsen, 1950*a*) which showed that the lung tissue of Street mice was susceptible to this dose of dibenzanthracene, less so to this dose of 9:10-dimethyl-1:2-benzanthracene, but failed to respond to benzpyrene and methylcholanthrene.

These recent experiments also showed that the thymus was particularly susceptible to direct application of 0·02 mg. of 9:10-dimethyl-1:2-benzanthracene and methylcholanthrene, less to the same dose of dibenzanthracene and least to the same dose of benzpyrene. The incidence of thymic tumours induced by the four hydrocarbons was therefore decreasing in the same order following injection of a small dose into the thymus (Rask-Nielsen, 1950*a*) and a large dose into the lung. The results cannot be compared with earlier experiments, since thymic tumours following a direct carcinogenic action on the lung or thymus have not been reported before, with the exception of a few instances of thymic lymphosarcoma observed after injection of methylcholanthrene into the lung (Esmarch, 1940*b*).

It is evident that the carcinogenicity of 9:10-dimethyl-1:2-benzanthracene for the interstitial connective tissue of the lungs differed from that of the other three hydrocarbons. 9:10-Dimethyl-1:2-benzanthracene proved to be non-carcinogenic for this tissue, whereas the carcinogenicity of the other three hydrocarbons increased in the same order as their carcinogenicity for the subcutaneous connective tissue following application of the same dose into this tissue (Rask-Nielsen, 1950*b*), i.e. in the order benzpyrene, dibenzanthracene, methylcholanthrene. Since 9:10-dimethyl-1:2-benzanthracene has proved to have a carcinogenic effect, though only rather slight, on the subcutaneous connective tissue (Rask-Nielsen, 1948, 1950*b*), the response of connective tissue to this hydrocarbon seems to vary according to its site. The cause can hardly be a more rapid absorption of the hydrocarbon from the lung than from the subcutaneous tissue, since the result would probably be a marked remote effect of the hydrocarbon, the development of leukaemia, as seen following subcutaneous application (Rask-Nielsen, 1948, 1949). But such remote effect was not observed. Another explanation might be given, namely that 9:10-dimethyl-1:2-benzanthracene might be transformed into non-carcinogenic substances at a rate which leaves time to affect the more susceptible tissues, the specific lung tissue and the thymus (Rask-Nielsen, 1950*a*), but not the interstitial connective tissue which is less susceptible to 9:10-dimethyl-1:2-benzanthracene. In that case, the other three hydrocarbons must be presumed to be transformed into non-carcinogenic products at a slower rate or to possess a higher carcinogenicity for the interstitial tissue of the lungs.

When applied by the technique used in the present experiments, all four hydrocarbons proved capable of inducing specific tumours of the lung and thymus. 9:10-Dimethyl-1:2-benzanthracene had a carcinogenic effect on the subcutaneous connective tissue, but not on the connective tissue of the lung, whereas the other three hydrocarbons proved carcinogenic for the subcutaneous connective tissue as well as for that of the lung. It will be seen, therefore, that the four hydrocarbons differed, not only as regards their relative carcinogenicity for the three tumour-producing tissues, but also as regards the order of this carcinogenicity for the three tissues. These findings accord with the results of corresponding experiments with small doses of hydrocarbon (Rask-Nielsen, 1950*a*) and show,

like the latter, not only that (1) various tissues in the same strain and even in the same mouse differ in their response to a given hydrocarbon, but also that (2) there is a difference in the response of each tissue to the same dose by weight of various hydrocarbons.

SUMMARY.

The object of the experiments reported in this paper was to study the carcinogenic potency of benzpyrene, dibenzanthracene, methylcholanthrene, and 9:10-dimethyl-1:2-benzanthracene, when injected directly into the lungs of Street mice in large doses of 0·5 mg.

Dibenzanthracene appeared to produce a higher increase in the development of pulmonary adenoma than did the other three hydrocarbons. Injections of benzpyrene, dibenzanthracene, methylcholanthrene and 9:10-dimethyl-1:2-benzanthracene were, moreover, followed by spindle-cell sarcoma of the lung, and, in a certain number of cases, spindle-cell sarcoma of the chest wall, diaphragm or mediastinum and lymphosarcomatous thymic growths.

In a discussion of the results it is pointed out that the carcinogenicity of the hydrocarbons for the three tumour-producing tissues differed and that the order of their relative carcinogenicity for the three tissues also differed.

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LOCAL AND REMOTE TUMOURS IN STRAIN STREET MICE FOLLOWING SUBCUTANEOUS INJECTION OF LARGE DOSES OF FOUR DIFFERENT CARCINOGENIC HYDROCARBONS.

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PREVIOUS investigations included experiments involving subcutaneous injection of the large dose of 0·5 mg. of 9:10-dimethyl-1:2-benzanthracene into the flank and below a nipple in strain Street mice (Rask-Nielsen, 1948). In both sites the injection induced only spindle-cell sarcomas and, in a few instances, squamous-cell carcinomas, but no mammary carcinomas. At the same time a marked development of leukaemia was observed, the leukaemic manifestations varying according to the site of injection. The ratio of cases of isolated thymic tumours to cases of generalized leukaemia was found to be 1 : 1 following injection into the flank and 1 : 5 following injection into the mammary region. Renewed experiments with subcutaneous injections of 0·5 mg. of 9:10-dimethyl-1:2-benzanthracene were performed. The leukaemic manifestations induced have been described in detail in a recent publication (Rask-Nielsen, 1949) and the local tumours will be dealt with in the present paper.

For the purpose of ascertaining whether the failing development of mammary carcinoma might be a phenomenon specific for injection of 9:10-dimethyl-1:2-benzanthracene, mice of strain Street were injected with 0·5 mg. of 3:4-benzpyrene, 1:2:5:6-dibenzanthracene, and 20:methylcholanthrene, into the mammary region. In an endeavour to find out also whether injection of these three hydrocarbons would induce equally marked development of luekaemia and whether the leukaemic lesions would differ in the same way as following injection of 9:10-dimethyl-1:2-benzanthracene, into the two sites, the three hydrocarbons, in doses of 0·5 mg., were injected subcutaneously in the flank as well. These experiments will be reported in the present paper which also includes the earlier experiments with 9:10-dimethyl-1:2-benzanthracene (Rask-Nielsen, 1948, 1949) for comparison.

MATERIALS AND METHODS.

Benzpyrene, dibenzanthracene or methylcholanthrene, 0·5 mg., was injected, suspended in 0·01 c.c. of a mixture of the same hard and liquid paraffin as used in the analogous experiments with 9:10-dimethyl-1:2-benzanthracene (Rask-Nielsen, 1948, 1949). As regards the two last-mentioned hydrocarbons, the same suspension was used for injection into the flank and into the mammary region, whereas a new suspension of benzpyrene had to be prepared from a new batch of benzpyrene for the injection into the mammary tissue.

The carcinogenic agents were injected into the left flank or below the second lowest nipple on the right side.

The mice used were of strain Street, ranging in age from 4 to 7 weeks. An equal number of females and males were injected into the flank, but only female mice were injected into the mammary region, except in a few 9:10-dimethyl-1:2-benzanthracene experiments, where an equal number of each sex was used.

More than half the experiments were carried out with litter mates, one half of each litter being left untreated as a control group. The remaining experiments were carried out without controls. Table I sets out the spontaneous tumours

TABLE I.—*Tumours Observed in the Controls.*

	Incidence.	Age in months.
Leukaemia	4/302, 1·3%	6, 8, 9, 14
Mammary carcinomas in non-breeding females	12/134, 8·7%	10, 16, 16, 17, 17, 18, 18, 19, 23, 24, 25, 26
Pulmonary adenomas	7/143, 5%	11, 16, 16, 17, 25, 25, 27
Subcutaneous sarcoma	1/22, 5%	23

observed in all the controls. It will be seen that the incidence of leukaemia was 1·3 per cent, of mammary carcinoma in non-breeding females 8·7 per cent, of pulmonary adenoma 5 per cent, and of subcutaneous sarcoma 5 per cent. These figures are in fair accordance with previous findings of spontaneous tumours in Street mice (Rask-Nielsen, 1948, 1949; Lefèvre, 1945).

RESULTS.

The number and survival time of the experimental mice are presented in Table II.

TABLE II.—*Number and Survival Time of Experimental Mice.*

Site of injection.	Injection of 0·5 mg.	Months. 3.	6.	9.	12.	15.	18.	21.	24.	27.	30.
Flank	Benzpyrene	63	36	27	17	12	9	3	3	2	0
	Dibenzanthracene	43	34	26	13	5	1	0	—	—	—
	Methylcholanthrene	57	27	9	4	4	3	2	1	1	
	9:10-Dimethyl-1:2-benzanthracene	306	152	44	20	2	1	0	—	—	—
Mammary region.	Benzpyrene	58	38	15	7	5	4	4	1	1	0
	Dibenzanthracene	53	17	17	9	6	4	2	2	0	—
	Methylcholanthrene	60	33	13	5	0	—	—	—	—	—
	9:10-Dimethyl-1:2-benzanthracene	345	164	40	12	2	1	0	—	—	—

Local tumours.

The local tumours were sarcomas and squamous-cell carcinomas as apparent from Table III. The former were of the ordinary type, usually spindle-cell

TABLE III.—*Incidence of Local Tumours.*

Site of injection.	Injection of 0·5 mg.	Spindle-cell sarcomas. Incidence.	Age in months.	Squamous-cell carcinomas. Incidence.	Age in months.
Flank	Benzpyrene	14/51, 27%	4–12	3/51, 6%	7–11
	Dibenzanthracene	18/36, 50%	7–15	—	—
	Methylcholanthrene	17/44, 39%	5–9	3/44, 7%	8–30
	9:10-Dimethyl-1:2-benzanthracene	26/222, 12%	4–10	2/222, 0·8%	5–7
Mammary region.	Benzpyrene	2/54, 4%	8–10	1/54, 2%	8
	Dibenzanthracene	11/50, 22%	8–18	1/50, 2%	27
	Methylcholanthrene	17/46, 37%	5–11	10/46, 22%	4–13
	9:10-Dimethyl-1:2-benzanthracene	21/254, 8·3%	5–9	6/254, 2·4%	4–8

sarcomas of a varying degree of differentiation ; a few were polymorphocellular, and a few rhabdomyosarcomas occurred. In the squamous-cell carcinomas marked cornification was a frequent finding ; no microscopic changes indicated that the growths might have originated in mammary tissue. On the other hand, 3 mice, 8, 11 and 13 months of age, exhibited typical mammary carcinomas in a site which made it impossible to exclude local tumour production. In view of the age of these mice, however, the growths were considered to be most probably spontaneous and were, therefore, not included in Table III.

Following injection in the flank, spindle-cell sarcomas were induced by benzpyrene, dibenzanthracene, methylcholanthrene and 9:10-dimethyl-1:2-benzanthracene in 27 per cent, 50 per cent, 39 per cent and 12 per cent of the effective total of mice respectively. Squamous-cell carcinomas were observed in the same experiment in 6 per cent, 7 per cent and 0·8 per cent following the injection of benzpyrene, methylcholanthrene and 9:10-dimethyl-1:2-benzanthracene respectively. As regards the application into the mammary region, spindle-cell sarcomas were observed following injection of benzpyrene, dibenzanthracene, methylcholanthrene, and 9:10-dimethyl-1:2-benzanthracene in 4 per cent, 22 per cent, 37 per cent and 8·3 per cent respectively. In this experiment, squamous-cell carcinoma was observed in 2 per cent, 2 per cent, 22 per cent and 2·4 per cent respectively. The effective total of experimental mice is the number of mice living to be as old as the youngest tumour-bearing mouse, namely 4 months.

The minimum, maximum and average latent period, the interval from the injection until death, is presented in Table IV. The average latent period of

TABLE IV.—*Minimum, Maximum and Average Latent Period of the Local Tumours (in weeks).*

Site of injection.	Injection of 0·5 mg.	Spindle-cell sarcomas.			Squamous-cell carcinomas.		
		Minimum.	Maximum.	Average.	Minimum.	Maximum.	Average.
Flank	Benzpyrene	15	59	32	27	46	35
	Dibenzanthracene	27	60	42	—	—	—
	Methylcholanthrene	16	35	22	29	127	65
	9:10-Dimethyl-1:2-benzanthracene	14	34	21	20	23	21
Mammary region	Benzpyrene	29	38	33	—	—	34
	Dibenzanthracene	31	74	47	—	—	101
	Methylcholanthrene	18	43	26	14	53	26
	9:10-Dimethyl-1:2-benzanthracene	13	36	24	11	28	18

spindle-cell sarcoma proved to be longest following injection of dibenzanthracene being 42 and 47 weeks following injection into the flank and the mammary region respectively. Following injection of benzpyrene, the latent periods were 32 and 33 weeks, following injection of methylcholanthrene 22 and 26 weeks, and following injection of 9:10-dimethyl-1:2-benzanthracene 21 and 24 weeks. Since squamous-cell carcinoma developed in such small numbers, the duration of the latent period is not applicable for deductions, but it appeared to be about the same as that of the spindle-cell sarcomas.

The marked difference in the incidence of tumours following injection of benzpyrene is presumably due largely to the fact that two different suspensions of the carcinogenic agent had to be used.

Considering the marked fluctuations in the incidence of new growths following

injection of small as well as fairly large doses of hydrocarbon under experimental conditions as identical as possible (Leiter and Shear, 1943), the present material probably justifies only the following conclusions :

(1) Under the experimental conditions concerned, neither benzpyrene, dibenzanthracene, methylcholanthrene, nor 9:10-dimethyl-1:2-benzanthracene was able to induce mammary carcinoma.

(2) The incidence of local tumours increased following injection of 0·5 mg. of the four hydrocarbons in the order : 9:10-dimethyl-1:2-benzanthracene, benzpyrene, dibenzanthracene, methylcholanthrene, and the latent period decreased in the order : Dibenzanthracene, benzpyrene, methylcholanthrene, 9:10-dimethyl-1:2-benzanthracene. In other words, the orders of the incidence and of the latent period of the tumours following injection of the four hydrocarbons were not the same.

Remote tumours.

Since non-local tumours developed to the same extent following injection into the flank and into the mammary tissue, the tumours of both experiments are set out together in Table V.

TABLE V.—*Incidence of Remote Tumours.*

Injection of 0·5 mg.	Leukaemia.		Pulmonary adenomas.		Granulosa-cell tumours.	
	Incidence.	Age in months.	Incidence.	Age in months.	Incidence.	Age in months.
Benzpyrene	7/105, 6·7%	4–21	1/74, 1·3%	25	—	—
Dibenzanthracene	1/86, 1·2%	24	1/51, 2%	14	—	—
Methylcholanthrene	0/90,	—	3/60, 5%	7–30	—	—
9:10-Dimethyl-1:2-benzanthracene	97/476, 20·4%	4–14	9/316, 2·8%	6–20	5/198, 2·5%	6–8

Leukaemia.—Leukaemia was observed following injection of benzpyrene, dibenzanthracene and 9:10-dimethyl-1:2-benzanthracene in 6·7, 1·2 and 20·4 per cent of the effective total respectively. Injection of methylcholanthrene did not induce leukaemia. The effective total is the number of experimental mice living to be at least as old as the youngest mouse affected with leukaemia, namely 4 months.

Among the seven instances of leukaemia observed following injection of benzpyrene, two were isolated thymic lymphosarcomas—occurring in mice aged 4 and 14 months. The remaining five cases, occurring in mice ranging in age from 14 to 21 months, were generalized leukaemias, characterized by an enlarged liver and spleen showing pronounced leukaemic infiltrations. One of the latter exhibited enlargement of the mesenteric lymph node ; in the others the lymph nodes were not enlarged. The average latent period of leukaemia was 63 weeks. In six cases the infiltrations were due to stem cells and in one to plasma cells. The only case of generalized leukaemia following injection of dibenzanthracene was of the former variety in a 24-month-old mouse.

The leukaemias observed following injection of 9:10-dimethyl-1:2-benzanthracene and the relationship of the leukaemic manifestations to the site of injection have been dealt with in a previous publication (Rask-Nielsen, 1949). It is therefore sufficient to state here that injection of this hydrocarbon into

the flank induced thymic tumours in 13 per cent and generalized leukaemia in 5·6 per cent of the females, whereas injection into the mammary region induced thymic tumours in 4·3 per cent and generalized leukaemia in 20 per cent of the females. In the males, no difference was apparent in the sites of the leukaemic manifestations. It appears from Table V that the total incidence of leukaemia following injection of 9:10-dimethyl-1:2-benzanthracene into the flank and into the mammary region was 20·4 per cent. The average latent period was 20 to 25 weeks (Rask-Nielsen, 1949).

The experiments thus go to show that the extensive and greatly accelerated development of leukaemia observed following subcutaneous injection of 0·5 mg. of 9:10-dimethyl-1:2-benzanthracene failed to appear following subcutaneous injection of the same amount of benzpyrene, dibenzanthracene, or methylcholanthrene. It is worth mentioning, however, that while dibenzanthracene and methylcholanthrene had definitely no effect on the development of leukaemia, it is impossible to rule out a faint, accelerating effect of benzpyrene, the incidence of leukaemia being 6·7 per cent as compared with an incidence of spontaneous leukaemia of 1 to 2 per cent. This accelerating effect, if any, caused only a slight increase in the incidence, but, unlike that following injection of 9:10-dimethyl-1:2-benzanthracene, no decrease in the latent period.

Pulmonary adenomas.—The study only includes macroscopic adenomas. In most instances there were only a couple or a few ordinary, typical, subpleural adenomas, about 1 mm. in size and in a few cases one or two larger swellings, 2 to 5 mm. in diameter, were found. Microscopical examination showed typical pulmonary adenomas sometimes with transitional stages to adenocarcinoma. In four cases the pulmonary adenomas co-existed with spindle-cell sarcomas, in two with leukaemia.

As apparent from Table V, pulmonary adenomas were observed following injection of benzpyrene, dibenzanthracene, methylcholanthrene and 9:10-dimethyl 1:2-benzanthracene in 1·3, 2, 5 and 2·8 per cent of mice respectively. Since the incidence of spontaneous pulmonary adenoma among the controls was 5 per cent (Table I) and since the incidence in previous experiments (Rask-Nielsen, 1948, 1949; Lefèvre, 1945) has been about the same, it is evident that injection of benzpyrene, dibenzanthracene, methylcholanthrene, and 9:10-dimethyl-1:2-benzanthracene failed to increase the incidence of pulmonary adenomas, but injection of methylcholanthrene and 9:10-dimethyl-1:2-benzanthracene appears to have accelerated the development of these tumours. For instance, four of the 12 mice with pulmonary adenoma in these two groups succumbed before the first control died with adenoma at the age of 11 months. It must be presumed, moreover, that the apparently low incidence of pulmonary adenomas is explicable to some extent by the comparatively short survival time of the experimental mice.

Finally, it should be mentioned that five cases of granulosa-cell tumours of the ovary were noted in mice aged 6 to 8 months among 198 mice injected with 9:10-dimethyl-1:2-benzanthracene and living to be at least 6 months of age, an incidence of 2·5 per cent. This type of tumour was not observed in any case following injection of the other three hydrocarbons.

On the whole, the experiments showed that injection of benzpyrene, dibenzanthracene and methylcholanthrene induced almost exclusively local tumours, the incidence increasing in the order mentioned. Injection of 9:10-dimethyl-1:2-benzanthracene did, indeed, induce a certain incidence of local tumours, but it

resulted in a much higher increase in the development of remote tumours, particularly leukaemia.

Mammary carcinoma was not induced by any of the four hydrocarbons.

DISCUSSION.

Local tumours.

Injection of the four hydrocarbons used in the experiments failed to induce mammary carcinomas. Previous attempts have given conflicting results. Injection of methylcholanthrene into certain strains of mice has induced these growths (Bonser and Orr, 1939 ; Strong and Smith, 1939 ; Strong and Williams, 1941 ; Strong, 1945), whereas injection into other strains has been ineffective (Strong and Smith, 1939 ; Esmarch, 1940*a*, 1940*b*). Injection of 9:10-dimethyl-1:2-benzanthracene has been reported to induce mammary carcinoma in one strain (dilute brown), but not in another (AKA) (Engelbreth-Holm and Lefèvre, 1941). Painting with methylcholanthrene has induced mammary carcinoma in some strains of mice (Engelbreth-Holm, 1941 ; Lefèvre, 1945 : Mider and Morton, 1939*a* ; Kirschbaum, Williams and Bittner, 1946), but not in strain Street (Lefèvre, 1945). Similarly, painting with 9:10-dimethyl-1:2-benzanthracene has accelerated the development of mammary growths in several strains (Engelbreth-Holm and Lefèvre, 1941 ; Lefèvre, 1945), but not in strain Street (Lefèvre, 1945). Apart from the presence of the milk agent and hormonal stimulation, the induction of mammary carcinoma, therefore, appears to depend more on the genetic constitution of the mice used than on the hydrocarbon. As far as the development of mammary carcinoma is concerned, Street mice appear to be refractory to painting with methylcholanthrene and with 9:10-dimethyl-1:2-benzanthracene as well as to injection of the four hydrocarbons used in the present experiments, whereas they have been found to respond to the action of oestrogens (Rask-Nielsen, 1948).

Some of the squamous-cell carcinomas observed in the present experiments may perhaps have originated in mammary tissue, although the microscopic architecture of the growths failed to indicate such an origin. If so, methylcholanthrene would appear to be particularly active in this respect, since the incidence of squamous-cell carcinoma following injection of this hydrocarbon into the mammary region was no less than 22 per cent.

The relative carcinogenicity of the four hydrocarbons for subcutaneous tissue estimated from the incidence of local tumours increased in the order, 9:10-dimethyl-1:2-benzanthracene, benzpyrene, dibenzanthracene, methylcholanthrene (Table III). Comparative experiments involving subcutaneous injection of 9:10-dimethyl-1:2-benzanthracene and other hydrocarbons under identical experimental conditions do not appear to have been published previously. In other experiments 9:10-dimethyl-1:2-benzanthracene has induced only relatively few subcutaneous growths (Shear, 1938 ; Engelbreth-Holm, 1939 ; Engelbreth-Holm and Lefèvre, 1941), a finding which is in keeping with the one reported in the present paper.

The relative carcinogenicity of benzpyrene, dibenzanthracene, and methylcholanthrene, on the other hand, was different from that usually reported (Greenstein, 1947), the carcinogenicity of dibenzanthracene having been reported to be lower than that of benzpyrene. In the present experiments the opposite order

was found. This difference must presumably be ascribed to a difference in the genetic constitution of the mice and possibly also to the vehicle used. The higher carcinogenicity of methylcholanthrene than of the other hydrocarbons apparent from the present experiments accords with previous findings (Greenstein, 1947). It accords also with the recent observation (Rask-Nielsen, 1950*a*) that subcutaneous application of 0·02 mg. of this hydrocarbon to Street mice induced local tumours in 10 per cent of the mice, whereas application of benzpyrene, dibenzanthracene, or 9:10-dimethyl-1:2-benzanthracene, 0·02 mg., failed to induce local growths. It is worth mentioning that the carcinogenicity of benzpyrene, dibenzanthracene, and methylcholanthrene for the interstitial connective tissue of the lung (Rask-Nielsen, 1950*b*), introduced directly into the lung in doses of 0·5 mg., increased in the same order as the carcinogenicity for subcutaneous connective tissue, whereas 9:10-dimethyl-1:2-benzanthracene failed to induce local spindle-cell sarcoma in the interstitial tissue of the lung. Accordingly, the connective tissue of the lung in Street mice at least, appears to respond differently to this hydrocarbon and to the other three agents. The order of carcinogenicity of the three hydrocarbons for the thymus was the same as in the case of the connective tissue, whereas the carcinogenicity of 9:10-dimethyl-1:2-benzanthracene for the thymus was practically the same as that of methylcholanthrene.

Remote tumours.

Table V shows that while injection of 0·5 mg. of 9:10-dimethyl-1:2-benzanthracene was followed by leukaemic manifestations in 20·4 per cent with an average latent period of 20 to 25 weeks (Rask-Nielsen, 1949), injection of dibenzanthracene and methylocholanthrene had no accelerating effect on the development of leukamia, and the doubtful, extremely faint acceleration observed following injection of benzpyrene affected at any rate only the incidence, not the latent period.

The absence of leukaemic lesions following injection of the three last-mentioned hydrocarbons is all the more remarkable, as the development of leukaemia has been found to be accelerated following painting with benzyprene in dilute brown mice (Morton and Mider, 1941) and in strain F mice (Kirschbaum and Strong, 1942) following painting with dibenzanthracene, without, however, decreasing the latent period in strain F mice and with methylcholanthrene in strain F mice (Kirschbaum and Strong, 1942), in dilute brown mice (Mider and Morton, 1939*b*; Morton and Mider, 1941; Lefèvre, 1945), in strains Rf, and Rf/Ak (McEndy, Boon and Furth, 1942), in C3H mice (Kirschbaum, Strong and Gardner, 1940; Morton and Mider, 1941), and in old and new Buffalo mice (Morton and Mider, 1941). Painting with 9:10-dimethyl-1:2-benzanthracene also accelerated the development of leukaemia in AKA mice (Engelbreth-Holm and Lefèvre, 1941; Lefèvre, 1945), in dilute brown mice (Engelbreth-Holm and Lefèvre, 1941; Law, 1941), and in Swiss mice (Law, 1941). No acceleration of the development of leukaemia has, however, been obtained by methylcholanthrene painting of C57 mice (Morton and Mider, 1941; Kirschbaum, Strong and Gardner, 1940) and of CHI, NH, CBAN mice (Kirschbaum, Strong and Gardner, 1940), or by 9:10-dimethyl-1:2-benzanthracene painting of C3H, Leaden, ABC, N and C57 mice (Law, 1941). Since painting, especially with methylcholanthrene, has proved to accelerate the development of leukaemia in several experiments

involving a number of different strains of mice, it seems strange that subcutaneous injection of this hydrocarbon had no remote effect, such as was observed following injection of 9:10-dimethyl-1:2-benzanthracene.

It was, however, reported by Lefèvre (1945) that the development of leukaemia in Street mice was accelerated only by painting with 9:10-dimethyl-1:2-benzanthracene and not by painting with methylcholanthrene. The difference in the effect of the two hydrocarbons found in the present experiments may, therefore, perhaps be ascribed to the genetic constitution of Street mice.

On the other hand, it is equally, if not more, probable that the difference in the remote effect of these hydrocarbons is partially or wholly due to their different solubilities and different rates of diffusion in the subcutaneous tissue. While subcutaneous injection of 9:10-dimethyl-1:2-benzanthracene induced leukaemic lesions in 20 per cent of the mice, the same dose of the hydrocarbon applied to the pulmonary tissue was followed by no such manifestations (Rask-Nielsen, 1950*b*). This finding would appear to support the latter explanation.

Another fact pointing in the same direction is the fairly extensive development of leukaemia following subcutaneous injection of 9:10-dimethyl-1:2-benzanthracene into AKA mice (Engelbreth-Holm and Lefèvre, 1941).

The same applies to the acceleration of granulosa-cell tumours noted in the present experiments following injection of 9:10-dimethyl-1:2-benzanthracene as compared with the failure of the other three hydrocarbons to accelerate this variety of growth.

The low incidence of pulmonary adenoma following subcutaneous injection of 9:10-dimethyl-1:2-benzanthracene may presumably be ascribed to the short survival time of the experimental mice (Table II). This explanation is also indicated by the occurrence of an increased number of adenomas in mice ranging in age from 11 to 26 months following injection of 9:10-dimethyl-1:2-benzanthracene, 0·02 mg. into the lung (Rask-Nielsen, 1950*a*), and by the induction of only microscopically visible adenomas in mice, 4–11 months of age, injected with 0·5 mg. of this hydrocarbon into the lung (Rask-Nielsen, 1948), whereas no grossly visible pulmonary adenomas were observed (Rask-Nielsen, 1950*b*).

To sum up, subcutaneous injection of benzpyrene, dibenzanthracene, and methylcholanthrene failed to induce remote tumours in Street mice, whereas corresponding injections of 9:10-dimethyl-1:2-benzanthracene accelerated the spontaneous development of leukaemia, pulmonary adenoma and granulosa-cell tumour, but not the spontaneous development of mammary carcinoma, subcutaneous, or cutaneous growths.

SUMMARY.

Subcutaneous injection of 0·5 mg. of benzpyrene, dibenzanthracene, methylcholanthrene, and 9:10-dimethyl-1:2-benzanthracene into the flank of strain Street mice induced local spindle-cell sarcoma in 27 per cent, 50 per cent, 39 per cent and 12 per cent ; and squamous-cell carcinoma in 6 per cent, 0 per cent, 7 per cent and 0·8 per cent respectively. Injection of the same hydrocarbons into the mammary region induced spindle-cell sarcoma in 4 per cent, 22 per cent, 37 per cent and 8·3 per cent and squamous-cell carcinoma in 2 per cent, 2 per cent, 22 per cent and 2·4 per cent respectively. Local mammary carcinoma was not induced.

Leukaemia developed following subcutaneous injection into the flank and into

the mammary region of benzpyrene, dibenzanthracene, methylcholanthrene and 9:10-dimethyl-1:2-benzanthracene in 6·7 per cent, 1·2 per cent, 0 per cent and 20·4 per cent respectively. The latent period was decreased only following injection of 9:10-dimethyl-1:2-benzanthracene. The development of pulmonary adenoma was not affected by injection of benzpyrene or dibenzanthracene, whereas injection of methylcholanthrene and 9:10-dimethyl-1:2-benzanthracene appears to have decreased the latent period of this growth.

Ovarian granulosa-cell tumours were noted in 2·5 per cent of mice following injection of 9:10-dimethyl-1:2-benzanthracene, whereas the other three hydrocarbons failed to accelerate the development of this growth.

The findings are discussed. The powerful remote, especially leukaemogenic, effect of 9:10-dimethyl-1:2-benzanthracene is presumed to be due to a more rapid absorption of this hydrocarbon from the subcutaneous tissue.

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EFFECT OF ACETAMIDOFLUORENE ON THE THYROIDS OF RATS TREATED WITH METHYLTHIOURACIL AND THYROXINE.

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In recent years several reports have been published describing the modifying influence of carcinogenic compounds on the action of hormones. Hertz and Tullner (1947) investigated the effect of simultaneous application of methylcholanthrene and testosterone propionate on comb growth in chicks. Flaks (1948) described inhibition of the action of chorionic gonadotrophin by methylcholanthrene. Paschkis, Cantarow and Stasney (1948) reported that " quantities of thyroxine adequate to prevent thyroid hyperplasia in rats receiving thiouracil alone did not inhibit hyperplasia in those receiving thiouracil and 2-acetamidofluorene (A.A.F.)." This observation, suggesting a disturbance in thyroxine metabolism, seemed important enough to justify re-investigation. By correlating thyroxine supply, cytology of the pituitary and functional activity of the thyroid in the manner described by Griesbach and Purves (1945), it was hoped to obtain a better understanding of the phenomenon reported by Paschkis, Cantarow and Stasney (1948).

METHODS AND MATERIAL.

Wistar rats of both sexes, 7 to 8 weeks old, were used. They received a diet consisting of whole meal flour (7 parts) and skimmed milk powder (3 parts), supplemented by cod-liver oil and cabbage once weekly. For each animal 12 g. of food were provided daily. The animals were divided into two groups. The rats of one group received 4 mg. of A.A.F. daily for the duration of the experiment. In the fourth week, methylthiouracil was added to the food of both groups, providing a daily supply of 5 mg. for each animal. The dose of methylthiouracil given to the males was doubled during the last 10 days before they were sacrificed. Four males and two females received methylthiouracil only ; two males and two females methylthiouracil plus A.A.F., and four females A.A.F. only. Thyroxine injections were started when the animals had stopped growing. This occurred in the females after three weeks of methylthiouracil treatment, in the case of the males, after four weeks. DL-Thyroxine was dissolved in saline with Na_2CO_3 to give a solution containing 10 μg. of thyroxine per ml. The amounts given were 2·5 μg. and 5·0 μg. per 100 g. body-weight, and were injected daily for 21 consecutive days. Twenty-four hours after the last injection the animals

were killed. During the period of thyroxine treatment the rats were weighed twice weekly and the injected amounts adjusted to the weights.

Five female rats, 7 to 8 weeks old, were thyroidectomized. Three weeks after operation, when the constant weight of the animals suggested completeness of thyroid ablation, these rats were started on the milk-flour diet containing 0·02 per cent of A.A.F. This concentration of A.A.F. supplied approximately 2 mg. of the carcinogen to each rat per day. Higher doses of A.A.F. are badly tolerated by thyroidectomized rats. After eight weeks of this régime, thyroxine treatment was started, each animal receiving one daily injection of 2·5 μg. of DL-thyroxine per 100 g. body weight for 21 days. During this treatment the A.A.F. diet was continued. All animals were killed with coal gas and their thyroids and pituitaries removed. The thyroids were weighed and then fixed in Acid-Zencker solution and cut at 5·0 to 7·5μ. The mean cell heights were measured as described by Griesbach and Purves (1943). The pituitaries were fixed in sublimat-formalin, cut at 2·5μ and stained according to Martins' (1933) modification of Mallory's method. In each pituitary 1500 to 2000 cells were counted under high power. The livers were weighed and those with macroscopical lesions were examined histologically.

RESULTS.

The animals which received A.A.F. only, for 10 weeks, had normal thyroids and pituitaries (Table I).

The male rats treated with methylthiouracil alone or in combination with A.A.F. had grossly enlarged hyperplastic thyroids and showed a marked basophilia of the pituitaries. The corresponding females which had not been separated from the thyroxine treated animals showed these changes to a slightly lesser degree (Table I).

TABLE I.—*Rats treated with A.A.F.* (1–4), *with A.A.F.* + *Methylthiouracil* (5–8), *and with Methylthiouracil* (9–14).

No.	Sex.	Thyroid weight (mg. %).	Mean acinar cell height (μ).	Pituitary cell counts (%).	
				Basophil.	Acidophil.
1	F.	10·0	7·8	8·6	44·7
2	F.	8·0	6·8	5·8	53·6
3	F.	8·8	8·0	8·6	51·6
4	F.	7·0	6·8	7·7	49·8
5	F.	44·9	12·5	19·5	9·3
6	F.	51·2	13·0	—	—
7	M.	53·0	14·3	26·7	0
8	M.	41·1	13·2	26·5	0
9	F.	41·4	12·6	20·2	9·5
10	F.	52·6	12·0	—	—
11	M.	30·0	15·3	38·2	0
12	M.	39·1	13·6	40·2	0
13	M.	32·7	11·9	36·4	0
14	M.	40·0	13·6	27·1	0

All animals (5 males and 6 females) treated with 5·0 μg. of DL-thyroxine per 100 g. body-weight for the last three weeks of the experiment had thyroids of normal or slightly increased size. The cell heights were reduced to subnormal values (Table II). The pituitaries showed low counts for basophil cells and, in

TABLE II.—*Female* (1–6) *and Male* (7–11) *Rats Injected with* 5 μg. DL-*Thyroxine.*

Methylthiouracil + A.A.F.

No.	Thyroid weight. (mg. %).	Mean acinar cell height (μ).	Pituitary cell counts (%). Basophil.	Acidophil.
1	12·4	3·9	4·6	52·6
2	14·8	7·7	3·9	49·9
3	13·2	—	3·5	46·9
7	9·7	5·4	4·0	61·8
8	10·8	5·2	6·0	57·5

Methylthiouracil.

4	13·3	4·7	5·7	57·0
5	12·7	4·0	5·5	59·3
6	9·1	4·7	5·3	53·8
9	8·1	3·1	—	—
10	10·0	3·9	5·9	64·2
11	8·7	4·0	6·5	69·5

TABLE III.—*Female* (1–16) *and Male* (17–22) *Rats Injected with* 2·5 μg. DL-*Thyroxine.*

Methylthiouracil + A.A.F.

No.	Thyroid weight (mg. %).	Mean acinar cell height (μ).	Pituitary cell counts (%). Basophil.	Acidophil.
1	14·3	5·9	6·2	52·1
2	34·5	11·4	8·0	45·1
3	43·3	12·9	8·4	41·1
4	12·4	11·4	7·7	51·6
5	14·3	8·9	10·8	50·1
6	28·2	11·6	9·4	43·9
7	28·5	12·1	5·3	51·8
8	16·2	12·7	5·7	54·0
Mean	23·96	10·86	7·69	48·75
St. Dev.	±10·63	±2·20	±1·81	±4·31
17	16·6	8·6	7·4	54·4
18	23·4	10·2	10·2	48·3
19	15·8	8·0	9·8	52·0
Mean	18·60	8·93	9·13	51·57
St. Dev.	±3·41	±0·93	±1·24	±2·51

Methylthiouracil.

9	9·3	4·8	6·6	48·5
10	19·3	5·5	6·1	52·1
11	15·1	5·7	10·3	51·3
12	21·3	8·6	11·0	48·3
13	15·3	7·9	9·7	45·4
14	19·9	7·3	5·6	48·2
15	9·4	4·9	4·4	51·1
16	15·8	6·4	5·8	46·3
Mean	15·67	6·39	7·44	48·90
St. Dev.	±4·23	±1·35	±1·98	±2·26
20	20·0	6·4	4·3	48·1
21	17·0	9·4	9·2	54·6
22	12·8	6·2	6·3	51·0
Mean	16·60	7·33	6·60	51·23
St. Dev.	±2·95	±1·46	±2·01	±2·66

the males, the number of eosinophils was increased above normal. These results were obtained in the rats fed a diet containing A.A.F. plus methylthiouracil, and also in those on methylthiouracil alone. Therefore, 5·0 μg. DL-thyroxine per 100 g. body weight completely compensated for the thyroxine deficiency produced by methylthiouracil, independent of the presence of A.A.F.

Twenty-two rats (6 males and 16 females) were treated with 2·5 μg. DL-thyroxine, a dose known to represent the daily requirement of thyroxine for our strain of rats. Neither the animals receiving A.A.F. nor the controls reacted as uniformly as the animals injected with the higher dose of thyroxine (Table III). In the males, the thyroid weights did not show any significant differences, the values being slightly above normal in both groups. A comparison of the acinar cell heights showed a tendency for higher values in the A.A.F. group. Of the 16 females, 8 received methylthiouracil and thyroxine, and served as controls. With two exceptions, the thyroid weights of these animals (No. 9–16) were above normal. The cell heights, however, were normal (6–8μ) or subnormal. The basophil counts gave six normal and two slightly increased values (over 10 per cent basophils). On the whole, as could be expected with a borderline dose, the controls showed a fair degree of variation with normal average values for cell heights and pituitary basophils and a slightly increased average for thyroid weights.

Much greater variations were seen in the eight females treated with A.A.F., methylthiouracil and thyroxine (No. 1–8). The thyroid weights ranged from slightly above normal to values as high as those of animals not treated with thyroxine. No colloid was present in the four largest goitres (Rats 2, 3, 6, 7, Table III). With the exception of two animals, all showed an increase in cell heights far above the normal values. All pituitaries were normal, showing basophil counts of the same order as the controls.

Comparison of the values obtained in the animals with and without A.A.F. shows that the greatest differences were found for the cell heights. Statistical analysis of these values proved that the difference between the means was highly significant ($P<0·01$). The difference found for the means of the thyroid weights of the two groups, however, was of doubtful significance ($P>0·05$).

The pituitaries of the five thyroidectomized females treated with A.A.F. plus 2·5 μg. of thyroxine showed a normal picture, the percentage of basophils ranging from 4·4 to 10·1.

The body weights of the A.A.F. treated males were lower than those of the controls and averaged 182 g. in the former, compared with 240 g. in the latter group. The corresponding values for the females were 160 g. and 173 g.

During the period of thyroxine treatment all animals gained in weight. The presence of A.A.F. in the diet reduced the increase of the body weight in the males only. Whereas the controls gained an average of 32·0 g. in 21 days, the male animals receiving the carcinogen gained an average of only 14·0 g. The corresponding figures for the females were 14·5 g. and 13·0 g. The five thyroidectomized females showed an average weight increase of 21·0 g. in 21 days of thyroxine treatment.

The livers of the A.A.F. treated females appeared macroscopically normal, with one exception (Table III, No. 8). The weights of these livers, however, were increased to an average of 4·7 per cent of the body weight, while the controls averaged 3·4 per cent. All the males treated with A.A.F. had grossly enlarged

livers which showed the typical picture of nodular hypertrophy with early cirrhotic changes. The average weight of these livers was 6·8 per cent, the control weights being 3·9 per cent of the body weight.

DISCUSSION.

For the proper assessment of the results obtained, an evaluation of the methods employed is necessary. The weight increase of the thyroid induced by goitrogens is a complex phenomenon. It is due to increased numbers and size of the epithelial cells, and may be partially offset by the loss of colloid. When the supply of thyrotropin is reduced either by withdrawal of the goitrogen, or by administration of thyroxine, cell size decreases within a short time, whereas, the number of cells remains high over prolonged periods and colloid reappears. A second factor influencing thyroid weight of activated glands is their hyperemia. The latter factor must introduce uncontrollable errors when the thyroid weight is used as a criterion of thyrotropic action. In our experience, as in that of Rawson and Starr (1938), the estimation of the mean acinar cell height has been found to give more reproducible results in assaying the degree of thyroid stimulation than the weight method. Since a close correlation exists between the size of thyroid cells and the number of pituitary basophil cells, the percentage count of basophils has been used for assaying states of thyroxine deficiency in combination with the measurement of epithelial cell heights (Griesbach and Purves, 1945). In the state of fully developed thyroxine deficiency the percentage of basophils reaches values of 30 to 45 per cent. When this deficiency is redressed by the administration of 2·3 to 2·5 μg. of DL-thyroxine, the number of basophils decreases to reach values of 10 per cent or less after 14 to 21 days when full compensation is achieved. At the state of regression it is sometimes difficult, in the stained pituitary section, to distinguish between basophil cells and colloid remnants of degenerated basophils. By excluding from the count masses of basophilic colloid free from cellular structures, we believe to have eliminated this possible source of error.

All rats injected with 5·0 μg. of DL-thyroxine reacted to the hormone treatment with complete reversal of thyroid hyperplasia and pituitary basophilia. The low values for cell heights and basophils are of the order found in animals after an overdose of thyroxine. This indicates that the thyroid and pituitary responded in an apparently normal manner to the higher dose of thyroxine (5·0 μg.). Paschkis, Cantarow and Stasney (1948) have published only the absolute amounts of thyroxine given to their rats. This makes it impossible to compare their dosage with that given by us. Further, these workers used L-thyroxine, whereas, in our experiments, DL-thyroxine was used. In addition, it may be pointed out that the amounts of thyroxine needed to prevent goitrogenesis, as in the experiments of Paschkis, Cantarow and Stasney (1948) may be lower than those required for involution of an established hyperplasia of the thyroid.

When 2·5 μg. of DL-thyroxine was given, a dose corresponding to the daily requirement of our rats, significant differences in response were found between the animals on A.A.F. plus methylthiouracil and those on methylthiouracil alone. Most of the female rats on A.A.F. had "active" thyroids in contrast to the controls, but the pituitaries of both groups showed a normal picture. As described by Griesbach and Purves (1945) and recently by Henegar and Higgins (1949), in

the goitrogen-treated rat enlargement of thyroid cells is linked with an increase in numbers of pituitary basophil cells, indicating elevated thyrotropic activity. This correlation breaks down under the influence of A.A.F. Whereas the pituitary changes induced by the goitrogen disappeared when 2·5 μg. DL-thyroxine was injected for three weeks, the thyroids were far less affected. One half of the animals still had large goitres and, histologically, in all but one the epithelium was almost as high as in the rats which did not receive thyroxine.

No evidence for an increased demand for thyroxine or for higher sensitivity to thyrotropin was found in the animals treated with A.A.F. alone. Their thyroids and pituitaries were normal. When a thyroxine deficiency was induced in A.A.F. treated animals by surgical or chemical means, their pituitaries responded in the same way to thyroxine as did the animals without A.A.F. Similarly, the growth response of the female rats to thyroxine was independent of the presence of A.A.F. In the males, the pronounced liver damage prevented normal growth.

These facts show that the action of thyroxine on the pituitary was not inhibited by A.A.F. The problem can therefore be reduced to that of an anomalous behaviour of the thyroid epithelium. In the hands of Paschkis, Cantarow and Stasney (1948) L-thyroxine did not prevent the change from normal to high epithelium. In our experiments, physiological amounts of DL-thyroxine did not reduce a high cylindrical epithelium to normal. It must, however, be remembered that the hyperplastic thyroid epithelium of the A.A.F. treated animals was not completely refractory to thyroxine. By doubling the amount injected to 5·0 μg., inactive thyroids were obtained. We have no evidence suggesting that factors outside the thyroid were responsible for this phenomenon.

SUMMARY.

Rats treated with acetamidofluorene and methylthiouracil responded to thyroxine in an anomalous manner.

With 2·5 μg. of DL-thyroxine injected for 21 days, the "thyroidectomy" pituitary was reconverted to its normal condition. This dose was insufficient to inactivate the thyroid which remained hyperplastic.

Under these conditions a dissociation existed between pituitary and thyroid.

The relative refractoriness of the thyroid cells must be attributed to the carcinogen acetamidofluorene.

The thyroid cells were inactivated by 5·0 μg. of DL-thyroxine in the presence acetamidofluorene.

We wish to thank Mr. T. H. Kennedy for supplying the solution of purified DL-thyroxine.

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THE ULTRAVIOLET ABSORPTION OF AQUEOUS SUSPENSIONS OF 20-METHYLCHOLANTHRENE.

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DURING the course of work on the effect of aqueous suspensions of 20-methylcholanthrene on protozoa it became evident that supposedly identical suspensions of the hydrocarbon varied beyond limits which could be attributed to preparation errors. For this reason it became necessary to standardize the material by some means. The usefulness of ultraviolet absorption in studying solutions of methylcholanthrene in different solvents and the physico-chemical significance of spectroscopic data suggested an approach to the standardization problem.

It has become necessary for us to discontinue work on the suspensions, and there is no likelihood of our returning to the matter. We feel, however, that our observations are of sufficient importance to warrant reporting in some detail.

MATERIAL AND METHODS.

A suspension of methylcholanthrene containing 2 to 3 μg. of hydrocarbon per ml. was prepared by adding an alcoholic solution of methylcholanthrene to boiling 0·01 per cent sodium chloride solution. Continued boiling, with adjustment of volume, reduced the alcohol to a final concentration of 0·002 per cent. Alcohol concentration was estimated from mass spectrometer studies.

After cooling, protected from atmospheric carbon dioxide, approximately one litre portions were distributed in each of 8 one litre glass cylinders. Column heights were made the same in all cylinders. Four of the cylinders were " protected " from carbon dioxide by long soda lime tubes; four were left " unprotected " except for a covering of tissue paper to prevent gross dust contamination, but allow a reasonably free diffusion of gases. Cylinders containing only 0·01 per cent sodium chloride were prepared in the same manner, and were used as controls in the comparison cell of the spectrophotometer.

Spectrophotometric determinations were made, using a Beckman spectrophotometer, on each of the cylinders immediately after preparation and again 2, 7, 21, 24, 58 and 80 days later. During the entire time all of the cylinders were exposed to the diffuse daylight of the laboratory.

Twenty mg. of methylcholanthrene crystals were ground to a paste with a few drops of 0·01 per cent sodium chloride solution. The wet paste was exposed, in a covered dish, to the diffuse daylight of the laboratory for 42 days. At the end of the exposure time the paste, which had been kept moist through the 42-day period, was extracted for 24 hours with 35 ml. of sodium chloride solution, and the absorption spectrum of the extract determined. Sodium chloride solution was used in the comparison cell.

For purposes of comparison, to be discussed later, the absorption of ·047 μg. of methylcholanthrene per ml. of alcohol was determined.

RESULTS AND DISCUSSION.

Significant differences between "protected" and "unprotected" suspensions were observed on the 7th day and showed a progressive increase in magnitude up to the 80th day. No suspensions were carried longer than 80 days. Curves serving to illustrate the differences in these two series are given for the 24th and 80th days only (Fig. 1, 2, 3, 4). These curves represent only one of the four suspensions carried in each series, since those subjected to like treatment gave similar results.

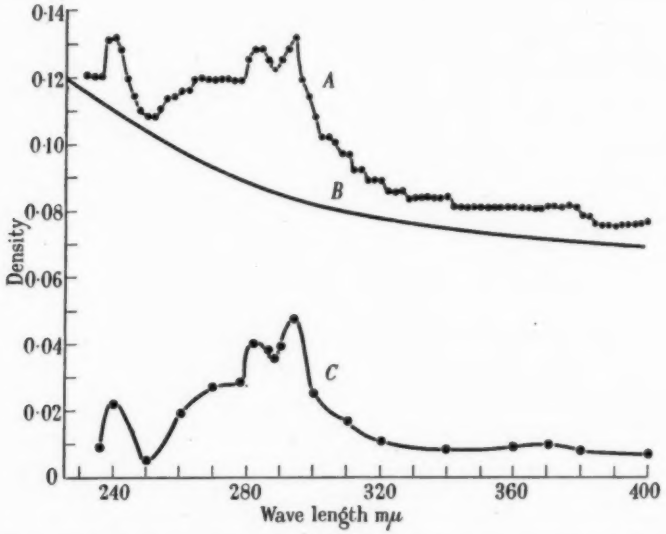

FIG. 1.—Ultraviolet absorption curves from a methylcholanthrene suspension, "protected" from atmospheric CO_2 after 24 days' exposure to diffuse daylight.

A. Curve as recorded from uncorrected spectrographic data.
B. Correction curve for scatter contribution.
C. Corrected absorption curve.

The A curves are to be compared with Fig. 6, which shows the absorption of 0·047 μg. of methylcholanthrene per ml. of alcohol. In alcohol methylcholanthrene is in true solution, and Fig. 6 represents the true absorption of methylcholanthrene in alcohol. All of the A curves (Fig. 1 to 5) show in addition to selective absorption a background density at all wave-lengths, with a tendency toward an increasing density at the shorter wave-lengths. This is the type of curve to be anticipated where in addition to true solutions there is a suspension of particles which will non-selectively remove light from the beam in the spectrophotometer.

Loss of light by reflection or diffraction from suspended particles is a strong

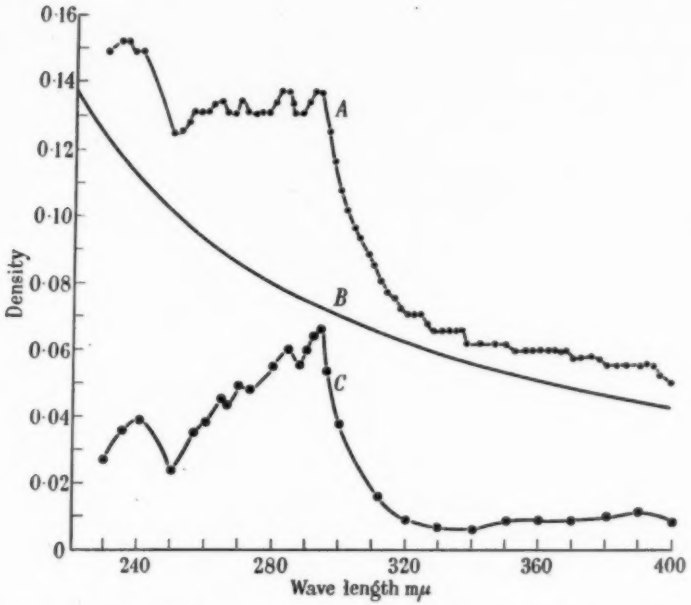

FIG. 2.—Ultraviolet absorption curves of an " unprotected " methylcholanthrene suspension after 24 days' exposure to diffuse daylight. Curves A, B, C as in Fig. 1.

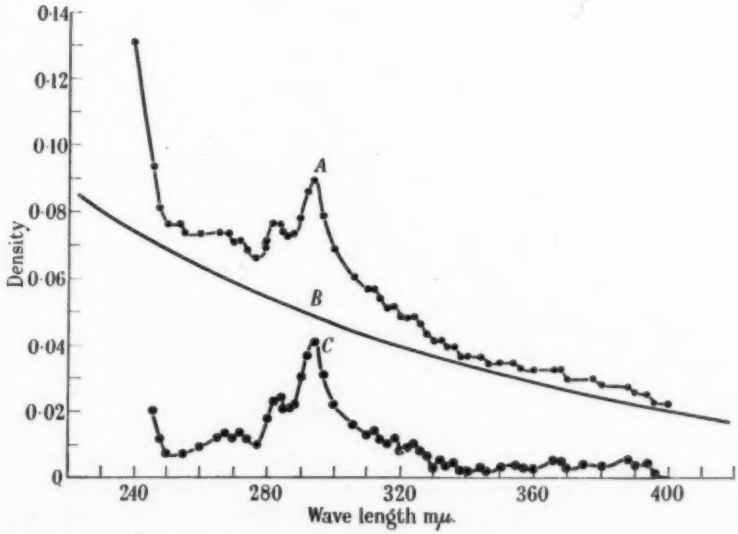

FIG. 3.—Ultraviolet absorption curves from a " protected " suspension of methylcholanthrene after 80 days' exposure to diffuse daylight. Curves A, B, C as in Fig. 1.

function of the size of the particles. With larger particles the effect is relatively constant with wave-length (turbidity), and for smaller particles the effect increases

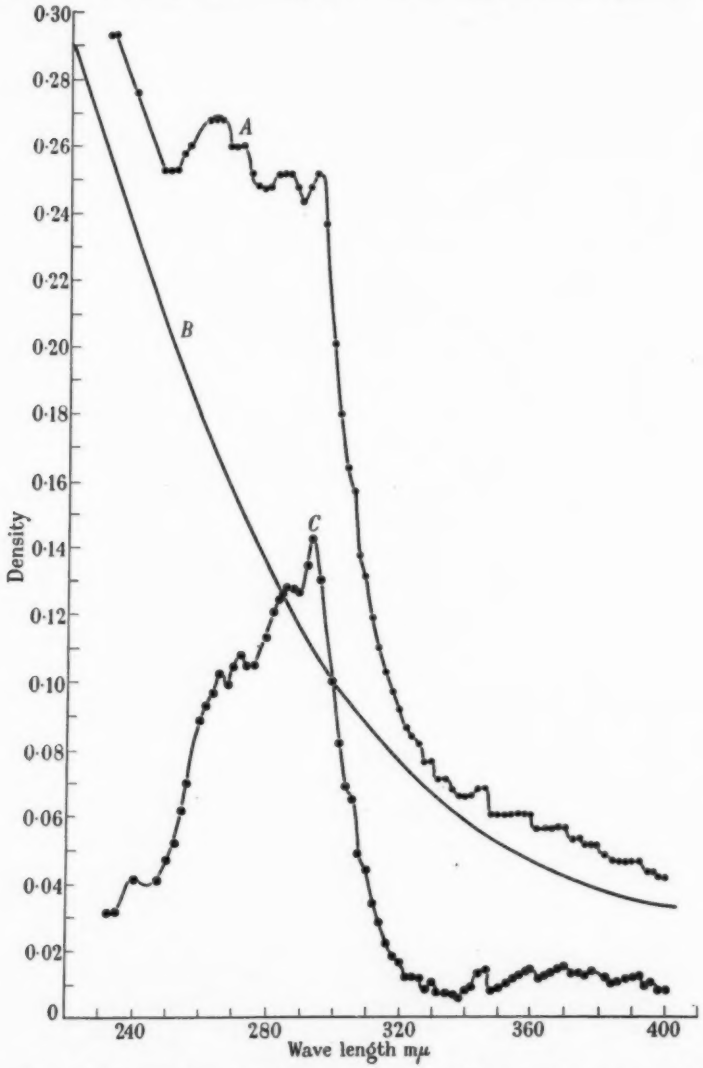

FIG. 4.—Ultraviolet absorption curves from an " unprotected " suspension of methylcholanthrene after 80 days' exposure to diffuse daylight. Curves A, B, C as in Fig. 1.

sharply with decreasing wave-length (scattering). In both cases, however, the variation with wave-length is smooth and quite distinct from the irregular varia-

tions due to absorption bands. For example, an inspection of A (Fig. 1) indicates a large contribution due to turbidity and a small contribution from scatter. In Fig. 4, A, the contribution of scatter predominates. It is evident that these solutions differ markedly in the type of suspended material, and this might well result in substantial differences in biological behaviour.

In order to arrive at a representative selective absorption curve the scatter component must be subtracted from the observed data. The scatter component may be reasonably estimated by a series of approximations which successively

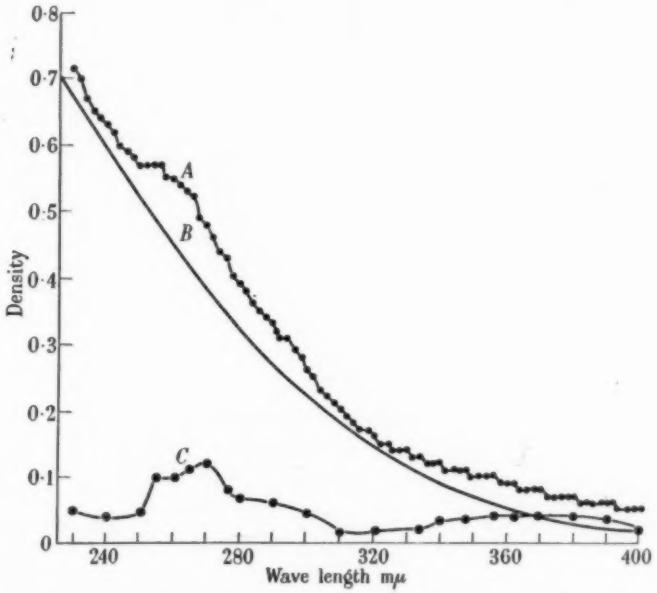

FIG. 5.—Ultraviolet absorption curves of a saline extract of ground, moist methylcholanthrene crystals which had been exposed to diffuse daylight 42 days before the extract was prepared. Curves A, B, C as in Fig. 1.

approach the actual contribution due to scatter. The procedure for making these approximations is as follows:

From an inspection of the curve plotted from the spectrophotometer data the contribution of scatter and turbidity is estimated and a smooth curve characteristic of scatter and turbidity is drawn to approximate this contribution. The choice of the initial correction curve is somewhat arbitrary, being based on experience as to the nature of such contributions. The accuracy of the location of the initial curve is not of great importance, except that a good estimate will reduce the number of approximations needed to secure an accurate correction. It is known that the initial curve will be below the points on the observed curve and nearest the points of least absorption; it will in no place cross the observed curve and will be smooth, continuously decreasing in value at the longer wavelengths.

The original spectrophotometer data must be plotted in terms of optical densities, since the correction method is essentially one of subtraction of scatter component and linear relations exist on the optical density scale, while they do not hold for transmission values.

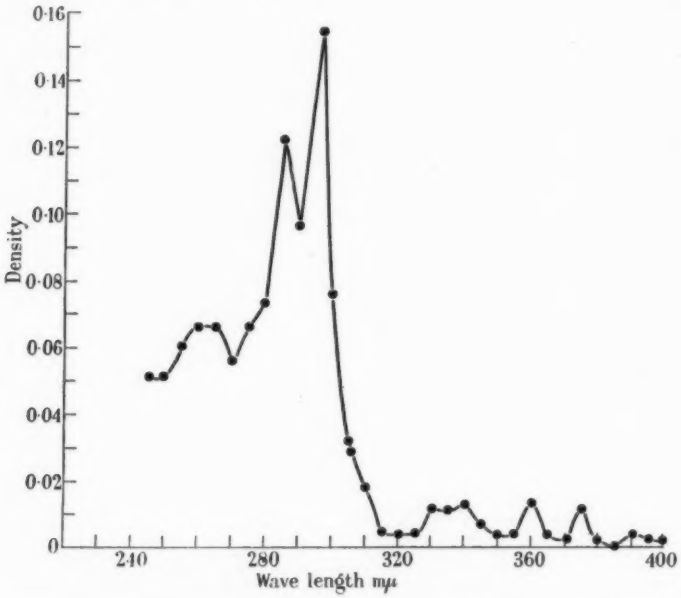

FIG. 6.—Ultraviolet absorption curve of an alcoholic solution of methylcholanthrene containing 0·047 μg. of hydrocarbon per ml. of alcohol.

The difference in optical density, at a wave-length of known high absorption of the solute, between the observed curve (the plot of the original spectrophotometer data) and the first scatter or correction curve is determined. (In the present instance 296·5 mμ. was used because methylcholanthrene has the greatest absorption at this point in both ethyl alcohol and iso octane). A high absorption value is used because the ratio of absorption to scatter at this point is higher than in those regions where absorption is least. The ratio of the difference obtained as above and the density value at the same wave-length of a known concentration of the solute dissolved in a solvent in which scatter is absent is determined. The ratio so found is used as a multiplying factor which is applied to the density values of the reference curve (known solute in true solution) at several wave-lengths. This product is subtracted from the density values at corresponding wave-lengths of the mixed scatter-absorption curve to give a series of density values which are plotted, and a smooth curve is fitted to the points so determined. By repeating the process as outlined, this time using the second approximation curve as the starting point, a third curve is determined

and plotted. Successive approximations may be made until a point is reached where the curves from two successive estimates will be almost superimposable. The difference between the best approximations and the mixed curve can be taken as a very reasonable estimate of the true absorption component of the original curve. The procedure as outlined gives a family of curves, with each member progressing towards a more accurate estimation and being uniquely related to the known absorption curve of the solute, and a mixed curve which is assumed to be composed of true absorption of the solute and a scatter-turbidity component.

Such a series of approximations can be made when one knows from other sources the true absorption spectrum of the solute, and can use this information as a standard of reference.

The above procedure has been used to determine the scatter curve presented in the figures of this paper. The curve shown is the second of two approximations.

The method of corrections for scatter components is not original with the authors. It is a statement of method which is in common use by spectroscopists, but does not seem to be available in published form. We are indebted to Dr. F. S. Brackett and Dr. H. L. Andrews of this Institute for their kindness in assisting us with the formulation and application of the procedure.

It is obvious from Fig. 1 and 3 that there is a gradual decrease in the turbidity of the suspension in " protected " cylinders, with no great increase in the scatter contribution. There is little or no change in the amount of material in solution between the 24th and 80th day.

In contrast to the " protected " series the " unprotected " series (Fig. 2 and 4) shows a very large increase in the scatter component and in the amount of material in solution.

Fig. 5 shows the absorption of the extract from the ground methylcholanthrene crystals. There is a large scatter contribution, otherwise the curve bears little resemblance to the curves from the suspensions. The entire curve may be due to scatter, although it is difficult to believe that no oxidation products were formed during the exposure to light of the wet crystals.

In the present study the particle size of the suspended material is not known. Furthermore, the reaction of the hydrocarbon with the several components of the suspension is an unknown factor. It is clear, however, that when a scatter curve, determined as outlined, is subtracted from the plot of the original spectrophotometric data, a curve results which approximates that of methylcholanthrene in alcohol.

There is obviously the choice of performing the experiments in the dark or in greatly reduced light intensity, or of working under conditions more nearly approximating those of the average biological laboratory. In view of the fact that the present work was not intended as an exhaustive study of photochemistry of the hydrocarbon, and since most experimental work is performed in a laboratory of average illumination, this alternative was chosen. It is generally accepted that methylcholanthrene undergoes photo-oxidation when exposed to daylight. The ground methylcholanthrene crystals (Fig. 5) should contain an appreciable amount of any water-soluble oxidation product produced from methylcholanthrene. There is, however, little resemblance between the curve of Fig. 5 and the curve for any of the suspensions. It is possible that the finely dispersed methylcholanthrene of the suspensions will give an oxidation product different from that produced in the mass of more granular material. So far as the authors

can learn there is no reason to assume the production of more than one photo-oxidation product from methylcholanthrene.

This paper shows that a suspension of methylcholanthrene in water is not a static system, but one in which complex physico-chemical changes are taking place without reaching an equilibrium over a period of at least 80 days. Furthermore, it does not seem likely that these changes are the result solely of photo-oxidation of the hydrocarbon. It seems probable that the method of preparing the suspension and the conditions of storage, including exposure to light, but not restricted to this factor, are of importance, and should be taken into account when the biological effects of aqueous suspensions of methylcholanthrene are being studied.

SUMMARY AND CONCLUSIONS.

Aqueous suspensions of methylcholanthrene exposed to diffuse daylight show spectral absorption curves indicating considerable contribution due to scattering and turbidity, as well as the contribution from the true absorption of the methylcholanthrene.

After correcting the scatter-turbidity contribution, the absorption curve of the suspensions of methylcholanthrene in dilute NaCl is similar to that of methylcholanthrene in alcohol.

In aqueous suspensions of methylcholanthrene exposed to diffuse daylight while unprotected from atmospheric carbon dioxide the scatter component of the curve increases with time. There is also an increase in dissolved material.

In aqueous suspensions of methylcholanthrene exposed to daylight while protected from carbon dioxide, there is no increase in scatter with time, and perhaps a slight decrease in soluble material.

A complex series of physico-chemical changes occurs in aqueous methylcholanthrene suspensions, and it is suggested that the nature and importance of such activity should be assessed in studies of the mechanism by which aqueous suspensions of the hydrocarbon influences a biological system.